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REPORTS ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

XXVII.

PRELIMINARY ACCOUNT OF PLANKTONEMERTES AGASSIZII, A NEW PELAGIC NEMERTEAN.

BY W. MCM. WOODWORTH.

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WITH ONE PLATE.

CAMBRIDGE, MASS., U. S. A.:
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Bulletin of the Museum of Comparative Zoology
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XXVII.

PRELIMINARY ACCOUNT OF PLANKTONEMERTES AGASSIZII, A NEW PELAGIC NEMERTEAN.

By W. McM. Woodworth.

[Published by Permission of Marshall McDonald and George M. Bowers, U. S. Fish Commissioners.]

With One Plate.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
July, 1899.

No. 1. — *Preliminary Account of Planktonemertes Agassizii, a new Pelagic Nemertean.* By W. McM. Woodworth.

Since the final report on the Nemerteans taken by the "Albatross" expedition cannot be ready for publication in the near future, owing to absences of the writer from Cambridge, the following brief preliminary account of a new pelagic Nemertean in the Albatross collections is now presented. No free swimming Nemertean has been described since the appearance of Moseley's[1] accounts of the two specimens of Pelagonemertes taken by the "Challenger." The Challenger material was afterward reported on in detail by Hubrecht,[2] and more recently Bürger,[3] in his great monograph, has made two distinct species out of the two specimens taken by the Challenger merely upon an examination of Moseley's sketches, basing his distinction upon the differences in the number of lateral diverticula of the intestine in the two specimens.

The form to be considered here, like Pelagonemertes, was taken in the Pacific Ocean, and in trawls from considerable depths. While the Challenger specimens came from latitudes well outside of the tropics, and from the western part of the Pacific, the specimens taken by the Albatross came from near the equator in the eastern part of the ocean. There are many points of resemblance between the two forms, resemblances in form, color, and even finer structure, which will not be discussed here, but a careful comparison with the detailed description by Hubrecht shows differences in structure of such fundamental importance that it has been necessary to establish a new genus in Moseley's family Pelagonemertidæ.

[1] Moseley, H. N. On Pelagonemertes Rollestoni, Ann. Mag. Nat. Hist., Vol. XV. p. 165, 1875. A Young Specimen of Pelagonemertes Rollestoni. Ibid., Vol. XVI. p. 377, 1875.

[2] Hubrecht, A. W. W. Report on the Nemertea collected by H. M. S. Challenger. Challenger Report, Zoölogy, Vol. XX., 1887.

[3] Bürger, O. Nemertinen. Fauna u. Flora Golfes von Neapel, Monographie XX., 1895.

Family PELAGONEMERTIDÆ Moseley.

Pelagic Nemerteans with a broad, flattened, leaf-like, gelatinous, very hyaline body. Rhynchocœlome extending nearly the entire length of the body. Proboscis unarmed. No cephalic grooves or organs of special sense. Intestinal tract dendrocœlous.

Genus PELAGONEMERTES Moseley.

Mouth and proboscis openings separate and distinct. Supraœsophageal ganglia larger than subœsophageal. Median dorsal vessel lacking. Lateral diverticula of the intestine comparatively few in number.

Genus PLANKTONEMERTES, nov.

A common external opening for the mouth and proboscis. Supraœsophageal ganglia smaller than subœsophageal. Median dorsal vessel present. Lateral diverticula of the intestine very numerous.

Planktonemertes agassizii sp. nov.

Five specimens were taken as follows: —

1. Station 3383, Lat. 7° 21′ 0″ N., Long. 79° 2′ 0″ W., 1832 fms. 6.51 A. M., March 8, 1891. Length 47 mm., greatest breadth 13.5 mm., greatest thickness 3 mm., color "orange." Figure 1.

2. Station 3361, Lat. 6° 10′ 0″ N., Long. 83° 6′ 0″ W., 1471 fms. 7.33 A. M., February 25, 1891. Length of body 24 mm., length of everted proboscis 28 mm., greatest breadth 9 mm., greatest thickness 2.5 mm., color "orange." Figure 2.

3. Station 3388, Lat. 7° 6′ 0″ N., Long. 79° 48′ 0″ W., 1168 fms. 6. 41 A. M., March 9, 1891. Length 14 mm., greatest breadth 5.5 mm., greatest thickness 1 mm., color "orange."

4. Same station as No. 3. Length 38 mm., greatest breadth 16 mm., greatest thickness 1 mm., color "orange." Figure 3.

5. Station 3406, Lat. 0° 16′ 0″ N., Long. 90° 21′ 30″ W., 551 fms. 6.47 A. M., April 3, 1891. Length 37 mm., greatest breadth 16 mm., greatest thickness 2 mm., color "pink." Figure 4.

The soundings given above indicate the depth at which the dredgings were made with an open trawl. The colors given in quotation marks are from notes taken by Alexander Agassiz on board the Albatross, and refer to the living animal. A water color drawing made by Mr. Agassiz of the living animal represented in Figure 4 shows the color as light brilliant scarlet, the intestinal diverticula and proboscis showing as bands of deeper color. The shape of the living animal as indicated by the sketch was like that of many of the larger marine Turbellaria, with parallel undular sides, and bluntly rounded at both ends.

All of the specimens except No. 2 (Fig. 2), which was not sectioned, proved to be females. In specimen No. 4 (Fig. 3) the ovaries were slightly developed, and could be seen only in sections. In the other three specimens the ovaries were prominent even before the specimens were subjected to a clearing reagent. Both specimens of Pelagonemertes taken by the Challenger were also females.

The chief differences between Pelagonemertes and Planktonemertes may be summarized as follows. In Pelagonemertes there are distinct openings for the mouth and proboscis, the former being ventral, the latter terminal; the dorsal cerebral ganglia are much larger than the ventral pair; the vascular system does not include a median dorsal vessel; the intestinal tract is comparatively simple, i. e. with few lateral diverticula, five and thirteen in the two specimens so far known. In Planktonemertes there is a common external opening for mouth and proboscis, slightly subterminal in position; the dorsal pair of brain ganglia are much smaller than the ventral pair; the vascular system includes a dorsal median vessel, which extends in the posterior portion of the ventral wall of the rhynchocœlom, and unites with the lateral vessels at the posterior end (this is seen in Figure 3); the intestine bears a great many (more than 50) lateral diverticula, and so profuse is the dendritic branching of these that in a transverse section the body appears to be honeycombed with cavities of varying size and outline, the gelatinous mesenchyma being but slightly developed.

A detailed study of this interesting form has already been made, but will be delayed in publication until the completion of the report upon the other Nemerteans taken by the expedition.

Museum of Comparative Zoölogy, Cambridge, Mass.,
May 10th, 1899.

EXPLANATION OF PLATE.

All Figures are of Planktonemertes agassizii.

The Figures were all of them drawn from unstained specimens cleared in oil of cloves.

All Figures magnified about $1\frac{3}{4}$ times.

1.

2.

3.

4.

W. McM. W. Del. Boston Eng. Co.

THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June, 1891, 16 pp. ; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates.

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893. 10 pp [Zool. Anzeig., No. 420, 1893.]

[6] Bull. M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z., Vol. XXV., No. 1 September, 1893, 25 pp.

[8] Bull. M. C. Z., Vol. XXV., No. 2, December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4, January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C. Z., Vol. XXV. No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV. No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z., Vol. XXV. No. 10, October, 1894, 109 pp., 12 Plates.

[14] Mem. M. C. Z., Vol. XVII. No. 3, October, 1894, 183 pp., 19 Plates.

[15] Bull. M. C. Z., Vol. XXV. No. 12, April, 1895, 20 pp., 4 Plates.

[16] Mem. M. C. Z., Vol. XVIII., April, 1895, 292 pp., 67 Plates, 1 Chart.

[17] Bull. M. C. Z., Vol. XXVII., No. 3, July, 1895. 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol. XXVII., No. 4, August, 1895. 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895, 14 pp., 3 Plates.

[20] Bull. M. C. Z., Vol. XXIX., No. 1, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M. C. Z., Vol. XXIII., No. 1, September, 1897, 92 pp., 15 Plates.

[22] Bull. M. C. Z., Vol. XXXI., No. 5, December, 1897. 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol. XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M. C. Z., Vol. XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, June, 1899, 4 pp., 1 Plate.

PUBLICATIONS

OF THE

MUSEUM OF COMPARATIVE ZOÖLOGY

AT HARVARD COLLEGE.

There have been published of the BULLETINS Vols. I. to XXXII.; of the Memoirs, Vols. I. to XXII.

Vols. XXXIV. and XXXV. of the BULLETIN, and Vols. XXIII. and XXIV. of the MEMOIRS, are now in course of publication.

The BULLETIN and MEMOIRS are devoted to the publication of original work by the Professors and Assistants of the Museum, of investigations carried on by students and others in the different Laboratories of Natural History, and of work by specialists based upon the Museum Collections.

The following publications are in preparation: —

Reports on the Results of Dredging Operations from 1877 to 1880, in charge of Alexander Agassiz, by the U. S. Coast Survey Steamer "Blake," Lieut. Commander C. D. Sigsbee, U. S. N., and Commander J. R. Bartlett, U. S. N., Commanding.

Reports on the Results of the Expedition of 1891 of the U. S. Fish Commission Steamer "Albatross," Lieut. Commander Z. L. Tanner, U. S. N., Commanding, in charge of Alexander Agassiz.

Contributions from the Zoölogical Laboratory, in charge of Professor E. L. Mark.

Contributions from the Geological Laboratory, in charge of Professor N. S. Shaler.

Studies from the Newport Marine Laboratory, communicated by Alexander Agassiz.

Subscriptions for the publications of the Museum will be received on the following terms: —

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Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
Vol. XXXV. No. 2.

THE ANATOMY AND PHYSIOLOGY OF THE MOUTH-PARTS OF THE COLLEMBOLAN, ORCHESELLA CINCTA L.

By Justus Watson Folsom.

With Four Plates.

CAMBRIDGE, MASS., U.S.A.:
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By Justus Watson Folsom.

With Four Plates.

CAMBRIDGE, MASS., U.S.A.:
PRINTED FOR THE MUSEUM.
July, 1899.

No. 2. — *The Anatomy and Physiology of the Mouth-Parts of the Collembolan, Orchesella cincta L.*[1] By Justus Watson Folsom.

INTRODUCTION.

The group Apterygogenea (Apterygota) of Brauer comprises two distinct groups, termed the orders Collembola (Lubbock) and Thysanura (Latreille). The Collembola, to which Orchesella belongs, have been comparatively little studied by naturalists, owing in part, no doubt, to their being small, unobtrusive, and delicate insects. It is not surprising, in view of the small size of the Collembola, that entomologists have described their mouth-parts imperfectly and very diversely; on the other hand, it is remarkable that so much good work has already been accomplished by dissection, unaided by the improved modern methods of technique.

An excellent résumé of preceding descriptions of the mouth-parts has been given by Lubbock ('73), to whose account, however, I shall make certain additions.

Fabricius (1777) briefly mentions the palpi, mandibles, maxillæ, and bifid labium of Podura, the genus in which all Collembola were originally placed. His description, especially inapplicable as regards the palpi, has been commented upon by Latreille and Lubbock.

Latreille ('32), who noticed the labrum, was hardly more successful than his predecessor. His account, quoted in full by Lubbock ('73), is characterized by the latter as being "vague as well as inaccurate."

Bourlet ('39), in a brief paragraph, also quoted by Lubbock ('73), adds "une languette large, saillante, ciliée, à deux divisions, chaque division quadrifide." Like Fabricius, he erroneously supposed that there were two pairs of palpi, to which he gave names.

Nicolet ('41), in his classical memoir, gives an extended and careful description of the most salient characteristics of the mouth-parts, recog-

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the Direction of E. L. Mark, No. XCVI.

nizing the anomalous condition found in Achorutes. Although a few of his interpretations are inaccurate and his drawings rather imaginative, Nicolet decidedly improved upon preceding authors, and his work is of great historical importance.

Lubbock ('62, '73) enumerates and figures the mouth-parts of Smynthurus, Papirius, and Tomocerus, recognizing "second maxillæ," which "are closely attached to the ligula." His clear descriptions are quite accurate, as far as they go, and have never been disputed. His elaborate elucidation of the body-muscles of Tomocerus and Smynthurus, which no subsequent worker has attempted to repeat, leads one to wish that Lubbock had extended his patient researches to the muscles of the head.

De Olfers ('62) describes the coarse anatomy of the mouth-parts; his account agrees in the main with Lubbock's. De Olfers notices a "lingua" and "organa cochleariformia," the confluent margins of the latter forming a tube, as expressed in the following passage: "Margines organorum cochleariformium confluentes ut supra diximus tubulum formant, quem œsophagum appellamus." He is mistaken, however, in terming this tube the œsophagus.

For the sake of completenesss, I allude to the work of Laboulbène ('65) upon the general anatomy of Anurida maritima, the mouth-parts of which differ widely from the type prevailing among Collembola.

Meinert ('65), in his noted paper upon Campodea and Japyx, gives important generalizations upon the mouth-parts of insects.

Packard ('71) offers several suggestions upon the homologies of the mouth-parts of Collembola.

Tullberg ('72), in an especially important contribution to the study of Collembola, describes and figures the skeletal portions of the mouth-parts of Tomocerus vulgaris in some detail, showing the general structure and more evident relations of the parts, and adding much to the meagre accounts of other authors. This author discovered and figured salivary glands in Tomocerus flavescens.

Sommer ('84) describes the muscles of the pharynx and œsophagus.

Oudemans ('87), in a valuable anatomical work, gives a convenient summary of the results of preceding investigators of the anatomy of Collembola.

Nassonow ('87) figures the salivary glands of Lipura [Aphorura] ambulans.

Von Stummer-Traunfels ('91), in the only article devoted exclusively to this subject, discusses the mouth-parts of Collembola and Cinura with

reference rather to their systematic value than to their anatomical detail. His accurate description, relating mainly to skeletal structures, is of great value.

Fernald ('90, '90[a]) maintained that the so called "salivary glands" of Anurida maritima opened into what Tullberg named the linea ventralis, a median ventral groove terminating in the ventral tube.

Willem and Sabbe ('97) accept the results of Fernald, and infer that the well known exudation from the ventral tube is secreted by the large cephalic glands, which had been regarded as salivary in nature.

Upon the whole, our present knowledge of the mouth-parts of Collembola is fragmentary and far from complete; it is indeed insignificant, compared with what is known of the mouth-parts of most other orders of insects. The physiological side of the subject is practically untouched.

Methods.

The mouth-parts of Orchesella cincta[1] were studied from dissections supplemented by serial sections. Dissections were necessarily made with needle-knives under dissecting or compound microscopes. First, the chitinous skeletons were prepared from alcoholic or fresh material by separating the mouth-parts in water and treating them with potassic hydrate. The immediate muscular attachments were then studied from dissections in water, weak alcohol, or glycerine, permanent preparations being made in glycerine. The normal relations of the mouth-parts were ascertained by rendering the entire head gradually transparent by weak potassic hydrate. At a certain stage in the process, the mouth-parts become prominent, owing to the pink or purple color they acquire from the solution and diffusion of the ectodermal pigment they contain. The stain thus obtained is permanent in glycerine or balsam dissolved in xylol.

For killing and fixing, preparatory to sectioning, the principal agents used were either hot water, hot alcohol, hot corrosive sublimate, or picro-sulphuric acid. Hot water or hot alcohol was as good as anything, causing a well relaxed condition; some of my best preparations were killed in hot alcohol of seventy per cent. Embedding was most conveniently per-

[1] The species used in this research has hitherto been called Orchesella flavopicta Pack.; it is, however, synonymous with the European O. cincta L., as I have ascertained by comparing Packard's types, which are preserved in the Museum of Comparative Zoölogy, with examples of the latter species given to me by Dr. C. Schäffer of Hamburg, by whom they were identified.

formed in watch-glasses. Serial sections of the head, from $3\frac{1}{3}$ μ to 10 μ thick, were cut with a Minot-Zimmermann microtome, not only in the three principal planes, but also obliquely. All sections were affixed to the slide with Mayer's albumen mixture, stained on the slide and preserved in xylol balsam. The most satisfactory of many staining methods tried was Kleinenberg's hæmatoxylin followed by safranin. Victoria green, like safranin, is a good stain for chitin. For differentiating nervous structures, good results followed the use of Vom Rath's picric-osmic-acetic mixture. For elucidating the structure of the glossa and paraglossæ, the useful device of reconstruction in wax from sections was used.

Mouth, Pharynx, and Œsophagus.

The external appearance of the mouth has already been described by previous authors, beginning with Nicolet; indeed, the figures of Tullberg and von Stummer-Traunfels are so accurate that my own figure (Plate **1**, Fig. 1) is unnecessary, except for completeness. The mouth in repose is tightly closed by the labrum, labium, and palpi, the palpi fitting snugly into apertures on either side of the labrum. When the insect is eating, the tips of the mandibles and maxillæ may be seen projecting a little from the mouth to grasp the food, but at other times they are concealed within the capacious pharynx. In no other order of insects is this curious protrusion and retraction of the jaws found.

The pharynx is evaginated to form four deep pockets, two on either side. In the dorsal and ventral pair of pockets are situated respectively the mandibles and maxillæ (Fig. 2). The glossa and paraglossæ are median in relation to the other mouth-parts (Fig. 3). The lower wall, or floor, of the pharynx is the dorsal surface of the labium, and its upper or anterior wall is formed by the labrum.

The œsophagus ascends abruptly from the antero-dorsal part of the pharynx (Figs. 2 and 3), but quickly bends directly backward to the middle of the mesothorax, where it terminates in a valve. The œsophagus is a slender tube of uniform calibre, except that the lumen of its anterior portion gradually enlarges from behind forwards.

The anatomy of the fore-gut has already been studied, especially by Sommer ('85), whose excellent account of Tomocerus applies also to Orchesella in most respects. There is a distinct intima of uniform thickness (Fig. 4, *i.*), which I have shown by chemical tests to be chitinous, and in some individuals this is furnished with numerous small and irregular teeth, as mentioned by Sommer. In other specimens, how-

ever, no such structures are observed, and, if they are not artificially produced, their presence may possibly depend upon the interval which has elapsed since the last moult, the intima being shed and replaced at every moult. The epithelial wall consists of a single, well developed layer of pigmented cells (Figs. 3 and 4, *e'th.*). The boundaries of the individual cells are not well indicated, but the arrangement of their large round or oval nuclei is an indication of their size. These cells, being doubtless of ectodermic origin, are, like the permanent "hypodermal" cells, pigmented; the pigmentation extends back through the whole length of the œsophagus to the stomach. The epithelium is thrown into four or five prominent longitudinal ridges (Fig. 4). A delicate hyaline and homogeneous basement membrane (*mb. ba.*) is distinguishable, surrounding which is a single layer of circular or constricting muscles (Figs. 3 and 4, *c'stt.*). Around the anterior portion of the œsophagus, which Sommer designates as pharynx, the muscle fibres are quite stout, but farther backward they gradually decrease in size, as do their nuclei also, and finally disappear near the base of the head. As there is but one nucleus to each circular fibre, the statement of Sommer ('85, p. 696) that "Jeder Ring entspräche hiernach vielleicht je einer Muskelzelle" is unnecessarily cautious. The nuclei of the circular muscles lie, as others have observed, in a single (median dorsal) line (Fig. 3, *nl.*), and each nucleus is contained in a loose sheath which is external to the muscle proper. The condition in Orchesella is so nearly identical with that of Macrotoma [Tomocerus] that I can apply Sommer's ('85, p. 691) description without change to the former species: "Die Muskeln zeigen eine zarte feinkörnige Aussenschicht, Perimysium, in welcher in ziemlicher Anzahl kleine runde Kerne eingelagert sind. Dieses Perimysium ist besonders mächtig an den Muskelbündeln des Kopfes entwickelt." There are no longitudinal muscles enclosing the constrictors. All the muscles in the head are conspicuously striated, and, in Sommer's ('85, p. 691) words, "Die im Körper sich findenden Muskelbündel sind von verschiedener Dicke, die einfachsten bestehen wohl aus einer einzigen Muskelfaser, während die stärkeren aus einer Anzahl von Primitivbündeln zusammengesetzt sind, wie man an Querschnitten leicht sehen kann. Die Insertion der Muskelbündel an die Chitincuticula erfolgt nicht unmittelbar, sondern mittelst einer Sehne."

The pharynx and œsophagus are dilated by means of thirteen pairs of muscles (Fig. 3, *dil.*), situated as follows. Four short muscles (Fig. 3, *dil. phy.*) originate on the clypeus in paramedian positions, and

immediately above the depressors of the labrum. The more dorsal pair pass directly backward, and are inserted side by side on the anterior wall of the pharynx (Plate 4, Fig. 29, *dil. phy.*). The other pair are similar, but longer, and are inserted considerably below the preceding. These four muscles evidently pull upon the toothed epipharynx, and serve to withdraw it from the teeth of the paraglossæ. On the dorsal side of the anterior part of the œsophagus are seven pairs of long slender muscles which originate on the front, and are affixed to the chitinous intima of the œsophagus (Fig. 3, *dil. œ.*). The members of each pair are widely separated in origin, but converge as they approach the œsophagus, penetrate between the circular muscles and epithelial cells, and are inserted dorso-laterally on the intima of the œsophagus by means of short spreading tendons. The posterior three muscles of either side unite to form a single head. Opposed to these dorsal muscles are four pairs on the ventral side of the œsophagus, which have a common tendinous origin on the anterior margin of the tentorium, and run forward under the œsophagus, to which they are affixed ventro-laterally in the same manner as the dorsal muscles. The function of these dorsal and ventral muscles is manifestly to enlarge the gullet; thus they are antagonistic to the circular muscles previously described.

Before describing the mouth-parts, it is best to consider an endoskeletal structure which is intimately concerned with them.

Tentorium.

The tentorium of Collembola has never been described; to dissect it out is extremely difficult; in potash preparations it is partially destroyed, and it is not easy, owing to its form, to make serial sections of it which will permit of accurate reconstruction. The failure to recognize the tentorium of Collembola as being the place of origin of the principal cephalic muscles, and homologous with the same structure in Orthoptera and other mandibulate insects, has led students to assign an altogether undue importance to the "Stützapparat" of the ligula, which has erroneously been regarded as a sort of substitute for a tentorium. Partly as a result of this error, systematists have acquired an exaggerated opinion of the differences which separate Collembola and Thysanura from insects of other orders.

The tentorium (Figs. 5 and 6) is a chitinized structure in the middle of the head, underlying the œsophagus, extending upward on either side of it, and held in place by three pairs of arms diverging from the median

plane. The ventral portion of the tentorium consists of a thin frontal plate (Figs. 5 and 7, *la. f.*), to the anterior margin of which are attached the ventral dilators of the pharynx (Figs. 3, 6, and 7), and under which may be seen certain of the muscles which adduct the mandibles (Fig. 7, *add. md.*). From the frontal plate diverge two anterior arms (Figs. 5 and 6, Plate **2**, Fig. 10, *br. a.*), which pass forward and downward, and become united with the paraglossæ (Plate **3**, Fig. 22, *br. a.*). The anterior arms bow outwards, and serve for the origin of the protrusors of the mandibles (Plate **2**, Fig. 14, *pr't.*). A second, or dorsal, pair of arms (Fig. 5, *br. d.*) diverge from the tentorium, and extend upwards on either side of the supra-œsophageal ganglion to the skull. Each dorsal arm is differentiated into two parts: a short proximal projection, which is part of the tentorium proper, and a long distal strand, less chitinized than the tentorium proper and distinctly fibrous in nature. As the strand shows no trace of cross striation and is chitinous, it can hardly be regarded as a muscle, but may be called a ligament. The third pair of arms project behind the tentorium (Figs. 5 and 6, *br. p.*). Each posterior arm curves downward, as well as outward, and consists distally of a ligament such as just described for the dorsal arms. The ligaments not only become continuous with the body of the tentorium, but also are securely attached to the heels (*cx.*) of the chitinous legs which support the ligula (Fig. 6).

The responses of the tentorium to stains and to potash prove it to possess three degrees of chitinization, the ligaments being least chitinous, the anterior arms strongly so, and the body of the tentorium intermediate in this respect. In preparations rendered transparent with potassic hydrate, whether subsequently stained with safranin or not, no trace of the tentorium is to be seen, except the anterior arms (Plate **2**, Fig. 10, *br. a.*) attached to the paraglossæ. When the tentorium is intact, the union of these arms with the rest of the endoskeleton is distinctly indicated by two curving sutures (Fig. 6, *sut.*).

The mass of muscles originating in the tentorium is at first bewildering; it is, nevertheless, possible to trace each muscle to its insertion, or, better, *vice versa*, and to infer its function. Having done this, I find no muscles which might protrude or retract the tentorium as Meinert ('65) claims for Japyx. This author (Meinert, '67, p. 367) says, "The opposite ends of the flexors of the mandibles, as well as of their tensors, in Japyx are attached to a chitinous plate situated between the mandibles, and steadied by a double set of muscles." The author figures the muscles referred to, and describes them as steadying,

protruding, and retracting the chitinous plate. The homologous muscles of Orchesella, however, and probably of other Collembola, I believe serve severally, not to move the tentorium, but to dilate the œsophagus (Fig. 6, *dil. œ.*), to move the antennæ, and to effect certain movements of the entire head (Fig. 6, *mu.*). The tentorium appears to be immovably fixed in place by means of the chitinous arms and ligaments already described. As I shall show, contrary to the views of other authors, the protrusion and retraction of the mandibles and maxillæ are accomplished, not by corresponding movements of either the tentorium or the "Stützapparat," but in both cases by special muscles.

I shall now describe the mouth-parts in the order of their position, passing from the dorsal toward the ventral side of the head.

Labrum.

The labrum is trapezoidal in external aspect (Plate **1**, Fig. 1, *lbr.*) and wedge-shaped in sagittal section (Plate **1**, Fig. 3). The external surface bears three transverse rows of stout bristles. Between the labrum and clypeus is a deep transverse suture, formed by the infolding of the chitinous cuticula, which becomes thin to form a hinge (Fig. 3). At either end of the hinge, however, the cuticula is swollen into a conspicuous chitinous lobe, which projects into the pharynx to fit against a corresponding prominence of the mandible. This relation between labrum and mandibles was expressed by de Olfers ('62, p. 12) in the following passage, which has been overlooked by subsequent writers: "Margo posterior [labri] incrassatus et formam literæ C imitans deorsum inflexus, qua re fit, ut duo apices in cavum oris promineant, qui mandibulas sustinent." The mandibles, when at rest, are held in place by these protuberances, and the surfaces against which the mandibles are applied show stout parallel ridges, which perhaps hold the mandibles effectively. Immediately behind the distal margin of the labrum are minute teeth projecting into the mouth in a transverse row, which becomes interrupted in the middle (Plate **2**, Fig. 9, *de.*). Between these submarginal teeth and the margin itself is a transverse groove, in which I have found the apex of the glossa locked by means of a corresponding transverse ridge (Plate **1**, Fig. 3).

Tullberg ('72, p. 20) discovered an epipharynx in Tomocerus vulgaris, and briefly described it in a sentence which I translate: "The pharynx is bounded above [anteriorly] by a palate, or epipharynx, which consists of a chitinous membrane furnished with several toothed elevations." The same structure also occurs in Orchesella (Plate **2**, Fig. 9, *e'phy.*). The

teeth of the epipharynx are directed towards those of the paraglossæ, in conjunction with which they appear to hold the food (Plate **4**, Fig. 30).

The only muscles within the labrum are dilators of the pharynx (already described) and depressors of the labrum. The latter consist of a pair of muscles (Plate **1**, Fig. 3, *dep.*), which originate on the lower margin of the clypeus in paramedian positions and converge downward toward the place of insertion, which is a chitinous ledge or shelf projecting inward from the anterior wall of the labrum. The contraction of these muscles doubtless closes the upper lip. I find no muscles which could conceivably elevate the labrum in opening the mouth. This being the case, the most satisfactory alternative which suggests itself is to assume that the external cuticula, which is bent like the letter S at the clypeo-labral suture (Plate **1**, Fig. 3), possesses an elasticity sufficient to raise the labrum when the depressors are relaxed.

The labrum is supplied by a pair of short nerves, which originate from the œsophageal commissures where the latter merge into the supra-œsophageal ganglion. The nerves soon ramify and become distributed between the hypodermal cells of the epipharynx and other regions. The labrum is lined with a single layer of deeply pigmented hypodermis cells with moderately large round nuclei (Plate **1**, Fig. 8, *h'drm.*). Near the base of the labrum and surrounding its central lumen, or body cavity, are grouped large oval nuclei (Fig. 8); each nucleus occupies the base of a filiform cell, which may often be traced directly to the base of one of the stout setæ (*set. sns.*) which cover the exterior of the upper lip. Sommer ('85, p. 703) regarded these in Tomocerus as sensory bristles, and the large oval nuclei as belonging to ganglion cells, although he gave little attention to the subject: "Was die Sinnesorgane betrifft, so muss ich mich darauf beschränken, dass sich eigenthümlich gestaltete Borsten, welche ich als Sinnesborsten bezeichne, an den Beinen, den Palpen, so wie der Ober- und Unterlippe vorfinden . . . sie stehen, wie ich das an denjenigen der Oberlippe direkt nachweisen konnte, mit Nervenfäden in Verbindung, welche aus einen Haufen von Nervenzellen hervortreten."

The so called "nerve-cells" have no direct connection with the central nervous system, however; they do adjoin a network of connective tissue (Fig. 8). I am disposed to consider the filiform cells as glandular in function, since they probably serve to produce the chitin of the sensory bristles. Deeper than the zone of large oval nuclei may be seen true ganglion cells, the nuclei of which are small and round, and in every

respect like those in the periphery of the brain, within the mandibles (Plate **2**, Fig. 17) and elsewhere. A delicate nerve fibre from each ganglion cell penetrates between the glandular cells and appears to accompany the filiform processes of the latter as far as the cuticula.

Mandibles.

In general form, the chitinous skeleton of either mandible (Plate **2**, Fig. 10, *md.*) is a modified, elongated hollow cone, a cross section of the least modified part being almost circular (Plate **4**, Fig. 31, *md.*). The specialized regions consist of an anterior or dental portion, and a posterior portion, named by Tullberg the fulcrum, for articulation and muscular insertions. The apex of the mandible bears several sharp, incisive teeth on its median side (Plate **2**, Figs. 10, 11), invariably five on the right mandible and four on the left in the many cases I have observed. Behind the apex, also on the median side, is an extensive convex molar surface (Figs. 10, 12) composed of minute raised teeth arranged in quincunx. This denticulated molar surface is bounded ventrally by a row of several large rounded conical teeth (*de. v.*). On the posterior end of the molar face is a single blunt tooth at right angles to the median plane of the head. At the base of the mandible is a conspicuous triangular medio-ventral opening (Fig. 15, *of.*) through which the large adductor muscles enter. Near the anterior angle of this aperture, on the median dorsal side is a conical projection (Fig. 10, *pr'j. con.*), serving for the insertion of a rotating muscle. Other muscles are inserted on the mesal face of a dorsal and oblique *basal ridge* (Fig. 10, *crs. ba.*). The extreme base of the mandible, a prolongation of its dorsal wall, is formed into a blunt pivot (Fig. 13, *cdx.*), upon which the mandible turns. This pivot is peculiar in that it does not form part of an ordinary articulation; it simply rests freely in a chitinous pocket or stirrup (Fig. 13, *sta.*), from which it is withdrawn when the mandible is protruded. The chitinous stirrup is formed from the cuticular lining of the cavity in which the mandible lies. The end of the stirrup against which the pivot bears when in motion is thickened, thus offering better resistance (Fig. 13, *cht.*). The pocket of the stirrup is fashioned from one extremity of an elongated, trough-shaped strap, the other end of which passes through the hypodermis and is continuous with the external cuticula of the head. I at first thought that the stirrup was free to swing forward and backward, carrying the mandible with it, but am now convinced that it is fixed in place and supports the mandible only when the latter is retracted.

Immediately behind the stirrup is a gland (to be described later) which may, as a secondary function, lubricate the pivot of the mandible.

The mandibles are situated in finger-like evaginations of the pharynx, and, except for muscular and nervous attachments, are unconnected with the pockets in which they lie, as is easily demonstrated in transverse sections of the mouth-parts. As von Stummer-Traunfels ('91, p. 220) observes, "Die mandibeln sind ganz frei in der Kopfkapsel gelegen, mit dem Stützapparate nur durch jener starken Muskel verbunden, und verdanken die Stellung, die sie einnehmen, nur noch dem Zuge der Kaumuskeln und einem Chitinvorsprunge an der Innenseite der Kopfkapsel, auf dem sie mit ihren hinteren Enden pivotiren und der diesem entgegenwirkt." It is evident that this arrangement facilitates the protrusion of the mandibles, which lie obliquely in the head (Plate **1**, Fig. 2, Plate **2**, Fig. 10), their bases close beside the skull on either side, while their apices converge, so that the opposing incisive teeth and molar surfaces meet in the sagittal plane. On account of the oblique position of the mandibles (Fig. 2), which in this agree with the maxillæ and tongue, it happens that microtome sections frontal or transverse in relation to the œsophagus are oblique in relation to these organs, and *vice versa*. Throughout this paper, when I refer to frontal and transverse sections, I shall use those terms with reference to the internal mouth-parts, conceiving the axial line between the mouth-parts to be their long axis, unless otherwise specified.

From the abundance of muscles in the head I have studied out the surprising number of ten distinct pairs which are concerned in moving the mandibles alone. This has been done by the study of serial sections in different directions and of dissections. The diverse directions taken by the muscles render them difficult to follow on sections in any single direction. On the whole, however, most may be learned from sections which are frontal, i. e. parallel with the plane in which the mandibles lie, and I shall describe the muscles as studied in successive frontal planes, beginning on the dorsal side. The order in which the muscles are numbered is necessarily somewhat arbitrary, but is chosen as being that in which they may with least difficulty be identified by any one who may wish to study the subject hereafter.

1. *Lateral Rotator.* This muscle (Plate **2**, Fig. 14, *1. rot. l.*) arises on the skull at the side of the head, passes forward and downward, crossing obliquely the dorsal surface of the mandible, and is inserted on the conical, medio-dorsal projection of the mandible. The same muscle is also represented as it appears in a transverse section of the mouth-parts

in Figure 16, *1. rot. l.* Its function evidently is to rotate the mandible in such a way as to raise the molar surface. The opposing muscle is designated as *3. ret. rot.*

2. *Abductor.* An extensive muscle (Fig. 14, *2. abd.*), which arises at the side of and anterior to the lateral rotator, passes obliquely forward and downward, and is inserted obliquely on the lateral surface of the mandible, which is excavated to receive the belly of the muscle. This is the only muscle which can act in opposition to the adductor (*9. add.*), and may also assist in the process of retraction.

3. *Retractor* and *rotator.* A slender muscle (Fig. 14, *3. ret. rot.*), which originates near the base of the skull in a dorso-lateral position, passes forward and downward, and is attached to the inner side of the basal ridge of the mandible described above.

4. *Retractor.* Although distinct from the preceding retractor, it follows nearly the same course (Fig. 14, *4. ret.*), but is more ventral; it originates nearer the median plane, and is inserted on the basal ridge immediately behind its companion. Both these muscles are adapted to withdraw the mandible into its socket after it has been protruded by muscles 5 and 6; No. 3, however, appears in addition to be the only muscle which is capable of rotating the mandible in opposition to Nos. 1, 7, 8, and 10, that is, so as to lower the molar surface.

5. *Lateral Protrusor.* The contraction of the outer of two slender cylindrical muscles (Fig. 14, *5. pr't. l.*) which originate on the anterior arm of the tentorium results in protruding the mandibles. It passes upward and backward from the tentorium along the mesal surface of the mandible, and is inserted immediately under the insertion of No. 3. Its function is doubtless to protrude the mandible by pulling its base forward.

6. *Mesal Protrusor.* This muscle accompanies No. 5 (Fig. 14, *6. pr't. ms.*), at the side of which it originates, but its course is more ventral, and its insertion is on the basal ridge just behind that of No. 5. The last two muscles are distinct, but have the same function, that of protruding the mandibles in opposition to muscles 3 and 4.

7. *Rotator.* A long stout muscle (Fig. 14, *7. rot.*), which begins on the side of a median dorsal chitinous projection near the base of the head. The muscle runs forward, outward, and downward, and terminates in a tapering pigmented tendon, which crosses under the base of the mandible (Fig. 15, *7. rot.*) and is inserted in the outer angle of the large triangular opening.

8. *Rotator.* A long, powerful muscle, which originates near the base

of the skull, being behind the preceding muscle and crossing the median plane (Fig. 14, *8. rot.*). It also ends (Fig. 15, *8. rot.*) in a tapering pigmented tendon, which is inserted close in front of the tendon of the last described muscle. Both muscles must act as rotators, twisting the mandible so that its molar surface moves upward and outward.

9. *Adductor.* This muscle, the only one hitherto mentioned by writers, is the most powerful muscle in the head. It originates principally on the tentorium (Fig. 14, *9. add.*), passes directly outward, penetrates the large triangular orifice of the base of the fulcrum (Fig. 15, *9. add.*), and is inserted on the inside of the lateral wall of the mandible. In addition, several of its fibres pass under the tentorium (Plate **1**, Fig. 7, *9. add. md.*) and become continuous with similar fibres from the opposite mandible. Thus, these fibres must pull against each other with the effect of closing the jaws, but with this exception the adductors are attached to the tentorium. These strong muscles are counteracted by muscle No. 2.

10. *Rotator.* A long, slender muscle, beginning at the median dorsal line, passing forward, outward, and downward, and inserted by a pigmented tendon along with Nos. 7 and 8. To avoid confusion I have omitted this muscle from Figure 14, but the figures of No. 7 (Figs. 14 and 15, *7. rot.*) will serve perfectly well for No. 10, if it be remembered that the latter muscle lies under the former. It will be observed that I have described as many as four muscles (Nos. 1, 7, 8, and 10) which appear to rotate the mandible in the same direction; I see no other function for these muscles, however. The rotation in the opposite direction seems to be comparatively unimportant, being accomplished by a single slender muscle (No. 3), the primary function of which is perhaps retraction. Among the mass of antennal muscles originating on the tentorium there is one which might easily be mistaken for a rotator of the mandible, in function similar to No. 3. This muscle (Fig. 16, *mu. at.*) in some sections is moulded against the mesal surface of the conical projection to which is inserted rotator No. 1. In other preparations, however, in which the mandible happens to have been rotated so as to remove the projection from its proximity to the antennal muscles, it may be seen that the apparent rotator is really unattached to the chitinous projection.

The nerves to the mandibles are the first pair of the infra-œsophageal ganglion; they arise from either side of the anterior part of the ganglion, pass directly outwards, enter the mandible at the anterior angle of its large lumen and extend the length of the mandible and into fine canals

in the chitinized apex. Each nerve fibre, soon after penetrating the mandible, becomes enlarged to contain a small round nucleus (Figs. 16 and 17, *cl. gn.*). The mandible is lined with a thick layer of deeply pigmented hypodermis (*h'drm.*), the confluent cells of which contain large oval nuclei (shown in cross section in Fig. 16), strongly contrasting in size with the nerve nuclei of the core of the mandible.

Maxillæ.

The maxillæ (Plate 3, Fig. 18) are about as long as the mandibles, and composed of two specialized regions: first, an anterior terminal movable lobe (Fig. 18, *cpt.*), which is subdivided into several smaller lobes and teeth; secondly, a posterior framework bearing muscles and supporting the terminal lobe and the palpus. The dorsal and outer portion of the terminal lobe is wholly chitinous and bears three stout, incurving claws (Fig. 19, *ga.*). This portion de Olfers ('62) compares with the galea of Orthoptera, and Packard ('71, p. 100), referring to the mouth-parts of Tomocerus plumbeus, writes, "The middle lobe, or galea, is nearly obsolete, though I think I have seen it in Smynthurus, where it forms a lobe on the outside of the lacinia." Von Stummer-Traunfels ('91, pp. 221, 223), however, says, "Es ist mir darum sehr zweifelhaft, ob dieser Theil des Kieferapparates die Deutung als äussere Lade wirklich verdient. . . . Bei Japyx noch zweifach gegliedert, ist er bei Campodea schon mehr reducirt und fehlt bei den Collembolen gänzlich." The careful comparative studies of the last mentioned author give much weight to his opinion. Underlying the tridentate lobe, or so called galea, are four chitinous lobes, or lamellæ, each of which bears on its inner margin a comb of fine teeth. Three of these lobes are falcate in form, and the fourth or inmost lobe (Fig. 19, *lcn.*) bears a prominent hook on its upper surface. These four fringed lobes probably represent the lacinia, or inner lobe, of other insects. The seven lobes and claws described appear to be firmly united basally, and, if so, cannot move separately, but must all move together by means of the articulation (Fig. 19, *atc.*) at the apex of the stipes. The movement is lateral only, and the adduction is accomplished by muscles which terminate in a slender chitinous rod (Figs. 18, 19, and 20, *bac.*) having the function of a tendon, and so named by von Stummer-Traunfels. This tendon is attached to the base of the inmost lobe of the lacinia.

Considering now the framework of the maxilla, the stipes (Figs. 18–20, *stp.*) is a stout chitinous structure, the form of which I may roughly

compare to a long, shallow boat, pointed at both ends and somewhat crescentic in transverse section (Plate 4, Figs. 29–32, *stp.*). The two thickened margins of the stipes are not parallel, however; the anterior portion of the dorsal margin is twisted toward the median plane of the head, as shown in Fig. 18. The ventral margin, moreover, is sharply incurved, as may be seen in transverse sections of the stipes (Plate 4, Figs. 31, 32). The anterior end of the stipes is rounded where it articulates (Fig. 19, *atc.*) with the movable head (*cpt.*) of the maxilla. The stipes is strengthened by a somewhat oblique cross rib connecting the two margins; the rib is prolonged beyond the dorsal margin as a free projection (Fig. 18, *pr'j.*), the function of which I am unable to state; I find no attachment of muscles or other structures upon it. The base of the stipes is immovably fixed to the cardo, the junction being indicated by a distinct suture (Fig. 18, *sut.*). The cardo (Fig. 18, *car.*) is shaped like a shoe, the toe of which is attenuated to form a chitinous ligament (Figs. 18 and 20, *lig.'*), which is continuous with a ligament from the foot of the glossa, a suture showing that the now single ligament originated from the union of two. By means of this peculiar articulation — already noticed by de Olfers, Tullberg, and von Stummer-Traunfels — and the ligament from the glossa to be next described are permitted the movements of the maxilla as a whole. The toe of the glossa, namely, is extended into a long flexible chitinous ligament (Fig. 10, *lig.*), which is fastened to the outside of the base of the stipes. The length of this outer ligament evidently determines the extent to which the maxilla may be protruded, the supporting stalks of the glossa being stationary.

The rod of chitin previously mentioned as assisting in the adduction of the head of the maxilla is crescentic in cross section (Plate 4, Figs. 29 and 30, *bac.*). Articulating with the base of the rod is a chitinous expansion (Fig. 18, *exp.*) for the insertion of four muscles. This expansion is nearly Λ-shaped in cross section (Plate 4, Fig. 31, *exp.*), there being a dorsal longitudinal ridge with a sloping wing on either side. In the angle between the ridge and the lateral wing is inserted muscle No. 7 of the maxilla. The opposite or mesal wing is prolonged backward in line with the rod (Figs. 18 and 20), and serves for the insertion of muscles Nos. 2 and 3. The base of the rod itself is prolonged into a short ligament (Fig. 18, *lig.*), by means of which the rod is connected with the adjacent corner of the base of the paraglossa (Fig. 18, *pa'gls.*). In order to show the ligament in Figure 18, I have represented the maxilla as withdrawn from the paraglossa. Normally, however, the

rod (*bac.*) is situated in a lateral concavity of the ligula (Plate 4, Figs. 29, 30, *gls.*), and consequently underlies the lateral margin of the paraglossa. The significance of the chitinized rod, or tendon, and its ligament, I shall presently show when describing how the head of the maxilla is abducted.

There are ten separate muscles which belong to each maxilla, excluding those of the palpi; like the muscles of the mandibles, they are most conveniently studied in frontal sections, although these must be supplemented by sections in other planes, as well as by dissections. The maxillæ are more complicated than the mandibles, and are correspondingly more difficult to understand; after long study, however, I have but little doubt as to their structure and movements.

1. *Retractor* and *abductor.* A long, slender muscle (Fig. 20, *1. ret. abd.*), which arises immediately beneath the origin of mandibular muscle No. 7 (Fig. 14) on the same median dorsal projection, and passes forward, outward, and downward to be inserted on the anterior concavity of the cardo (cf. Fig. 18). This muscle appears to retract and slightly abduct the entire maxilla by pulling the cardo backward. The retraction must be of slight amount, however, as the two ligaments which attach the cardo to the foot of the glossa prevent any extensive retraction or protrusion.

2 and 3. *Adductors.* Two cylindrical muscles, distinct from each other but lying side by side (Fig. 20, *2. 3. add.*, Plate 4, Fig. 32), which arise on the dorso-lateral part of the skull, pass forward and downward under the adductors of the mandible, and by means of several slender tendons fuse with the posterior elongation of the chitinous expansion just described (Fig. 20, *exp.*). I believe their function is, in co-operation with muscles Nos. 4 and 7, to adduct the head of the maxilla. In Figure 20, muscles 2 and 3 are represented as interrupted, in order to show certain underlying muscles.

4. *Adductor.* A stout muscle (Fig. 20, *4. add.*), which arises on the most anterior surface of the cardo, passes forward and is inserted on the ventral surface of the chitinous expansion.

5. *Protrusor* and *adductor.* A slender muscle (Fig. 20, *5. pr't. add.*), which begins on the side of the tentorium, goes outward and somewhat backward, and is attached to the anterior concavity of the cardo, just beneath the insertion of muscle No. 1.

6. *Protrusor* and *adductor.* Similar to the last in origin and direction, but more ventral and anterior in position (Fig. 20, *6. prt. add.*) and inserted on the most anterior surface of the cardo, just under the insertion

of No. 4. Muscles Nos. 5 and 6 both appear to have the same function, i. e. to protrude and adduct the entire maxilla by pulling upon the cardo. Protrusion may evidently take place until the outer ligament (Plate 2, Fig. 10, *lig.*) has become tense, whereupon additional contraction would produce adduction of the maxilla.

7. *Adductor.* A stout mass of muscle fibres within the maxilla (Fig. 20, *7. add.*) arising along the base of the stipes and inserted on the lateral aspect of the chitinous expansion (Plate 4, Fig. 31, *7. add.*). This large muscle passes under the free dorsal projection of the stipes, to which it does not appear to be fixed. The four muscles numbered 2, 3, 4, and 7, which are all attached to the chitinous expansion, which in turn is articulated with the chitinous tendon (*bac.*) from the head of the maxilla, probably adduct the head of the maxilla by retracting the rod, so that the claws of the head are rotated in a frontal plane toward the claws of the opposite maxilla, in order to meet them and grasp food; this function of the maxillæ seems to be the most important one, judging from the number and size of the muscles which close the claws.

8. *Retractor* and *adductor.* A short muscle on the ventral side of the maxilla (Fig. 21, *8. ret. add.*), its course also shown faintly in Figure 20, arising on the stalk of the glossa, passing obliquely forward and outward, and inserted on the inflexed lower margin of the stipes. This muscle must retract the stipes and draw it toward the median plane.

9. *Protrusor* and *adductor.* A broad, oblique muscle (Fig. 21, *9. pr't. add.*) beneath and running at right angles to the last, also arising on the stalk of the glossa and inserted on the upturned border of the stipes. By this muscle the stipes is protruded and also drawn towards the glossa.

10. *Adductor.* A short, stout muscle (Fig. 21, *10. add.*, Plate 4, Fig. 31) passing directly outward from the stalk of the glossa to the inflexed margin of the stipes. The function of this muscle is clearly to pull the stipes towards the glossa, i. e. to adduct it.

I believe that the three muscles last described have the important function of abducting the head of the maxilla, or separating the claws of the maxillæ preparatory to grasping food. The head of the maxilla is adducted by muscles numbered 2, 3, 4, and 7, which, by retracting the rod, cause the head to rotate upon the end of the stipes. The reverse movement, the opening, appears to be accomplished by the successive retraction and adduction of the stipes by means of muscles Nos. 8, 1, and 10, during which the head rotates upon the end of the chitinous rod until in the position shown in Figures 18 and 19. In this condition the

rod has evidently been pulled back, as far as its ligament (Fig. 18 *lig.*) allowed, by the action of the adductors of the head, and must manifestly be thrust forward before it can again be withdrawn. This protrusion of the rod appears to be a necessary result of the simple protrusion of the stipes, by muscles Nos. 9, 5, and 6; the advancing stipes pushes forward both the head and the attached chitinous rod until the ligament of the latter has become tense in the opposite direction.

The above explanation of the movements of the terminal portion of the maxilla is the only one I can offer, after long study. It appears reasonable to me, and accounts for the presence of the peculiar chitinized tendon or rod of the maxilla, as well as the unique ligamentous connection between the maxilla and paraglossa. I shall again refer to this connection when describing the palpi.

The maxillæ are supplied by the second pair of nerves from the infra-œsophageal ganglion; each nerve begins at the side of the ganglion, a little behind the mandibular nerve, goes directly outwards for a short distance and enters the maxilla at the posterior end of the chitinous expansion; a branch is soon given off to the palpus. The anterior portion of the maxilla is occupied by a core (Fig. 19, *gl. et n.*) of filamentous cells which penetrate into the lobes of the lacinia through an orifice in the base of the head of the maxilla; these filamentous cells are of two kinds and precisely similar to those already described for the labrum, the base of certain cells containing a large oval nucleus (Fig. 19), while intervening cells are ganglionic, with small round nuclei. The maxilla is lined with a single layer of confluent hypodermis cells, deeply pigmented and containing round nuclei of moderate size.

Palpi.

The palpi, consisting of but one pair, are of special interest because von Stummer-Traunfels considered them quite anomalous in position, having described and figured them as separated from the maxillæ and joined to the paraglossæ. This author ('91, p. 226) emphasizes, "Die grosse Unwahrscheinlichkeit, dass der sogenannte Maxillartaster der Collembolen wirklich zur Maxille gehört, indem diese von jenem vollständig getrennt ist und derselbe vielmehr in innigem Verbande mit der Paraglossen steht." This view originated, however, with Tullberg, as his description and figure show. I believe that this view is erroneous, and that the palpi unquestionably belong to the maxillæ; I shall show how the mistake mentioned might easily be made.

Each palpus (Fig. 18, *plp.*) is finger-shaped and composed of but a

single segment. The extremity is provided with five bristles, each seated upon a tubercle, the proximal bristle and its tubercle being much the largest. The palpus lies dorsal to its maxilla and its base is attached to the chitinous expansion of the maxilla, as represented in Figure 18. It will be noticed that the palpus joins the expansion close to the ligament which unites the chitinous rod (*bac.*) and the paraglossa (Fig. 18, *pa'gls.*), so that the ligament might readily be mistaken for a prolongation of the palpus. Careful study shows, however, that the ligament is directly continuous with the rod, and not with the palpus. If the palpus is connected with the paraglossa at all, the attachment is only of the most incidental nature.

The palpus contains at least two longitudinal muscles, which arise from the chitinous supporting structure at its base and extend to its free extremity. The palpus is lined with confluent, deeply pigmented hypodermis cells; beneath the setæ are filiform cells, each with an enlarged base which contains a large oval nucleus; a condition also found in the labrum and labium.

Glossa and Paraglossæ.

These structures have been briefly mentioned and differently named by several authors. By de Olfers ('62) they were called respectively *lingua* and *organa cochleariformia;* by Lubbock ('62), *ligula* and *second maxillæ;* by Meinert ('65), *lingua* and *paraglossæ;* by Tullberg ('72), *lamina hypopharyngis inferior* and *laminæ hypopharyngis superiores*; finally, by Grassi, Oudemans, and von Stummer-Traunfels, *ligula* and *paraglossæ*. Careful comparison has led me to believe that the ligula and paraglossæ of Thysanura are the equivalents, respectively, of the glossa and paraglossæ of other mandibulate insects; the Thysanura, however, exhibit the more primitive or generalized condition of tongue, which is not consolidated with the labium. The term ligula, at present, is properly applied to the glossa and paraglossæ taken together, rather than to the glossa alone.

The *paraglossæ* (Plate **3**, Fig. 22, *pa'gls.*) are two membranous, transparent, chitinous appendages attached to the dorsal surface of the glossa. The basal half of either appendage is firmly united to the glossa, and therefore can have no power of independent movement. The apical half is a free lobe, oval in cross section (Plate **4**, Fig. 28, *pa'gls.*). A paraglossa viewed from above presents somewhat the form of an isosceles triangle with a rounded apex and nearly equalling the glossa in length. The lateral surfaces are strengthened by being thicker and

more rigid (Fig. 22, *cht.*). The inner margins of the two appendages approximate each other, leaving an elliptical aperture however (Fig. 22, *lu.*), beneath which may be seen a single median row of fine teeth belonging to the dorsal surface of the glossa (Fig. 22). The inner edge of either paraglossa is furnished with teeth along its posterior half. Considering the teeth successively, the most anterior are blunt and lie in a frontal plane; as we pass backward the teeth are not only longer and more slender, but, as they approach the median plane, also become gradually erect, so that at length the posterior teeth project in a parasagittal plane. This change in direction is brought about by a curvature of the edge of the paraglossa, the dorsal surface of which, in passing backward, gradually becomes more concave, causing the inner margin to curve upward, or forward, as the parts naturally lie. The concavity of the paraglossa matches the convexity of the molar surface of the mandible on the same side of the head, and the sagittal teeth of the paraglossæ normally intervene between the grinding faces of the mandibles (Plate **4**, Fig. 30, *md.*).

I find no well marked bundle of nerve fibres for the glossa and paraglossæ, but many separate fibres, each from a ganglion cell, are given off directly from the infra-œsophageal ganglion and penetrate between the hypodermal cells of the tongue, which are for the most part attenuated like the filamentous chitin-forming cells of the labrum.

The *glossa* (Plate **3**, Fig. 23), situated under the paraglossæ which it bears, is an elongated, unpaired, chitinous organ, the median furrows of which however probably indicate its derivation from a paired condition. Three regions may be distinguished: a terminal, free portion (Plate **1**, Fig. 3, *gls.*), an intermediate region with which the paraglossæ are fused, and a basal part, consisting of a pair of supporting stalks (*pd.*, Plate **2**, Fig. 10, Plate **3**, Figs. 22, 23). The free portion is oval in cross section (Plate **4**, Fig. 28, *gls.*); viewed from above (Plate **3**, Fig. 23) it terminates in front in an oval transparent lobe, across the end of which is a subterminal dorsal fold or ridge (Fig. 23, *pli.*), which interlocks with the labrum (Plate **1**, Fig. 3). The upper surface of the terminal lobe is provided on either side with a curving row of minute teeth (Fig. 23, *de.*) borne upon a thickened chitinous ridge. A median dorsal groove is present, in the course of which occurs what appears to be an opening (Fig. 23, *of.*) into the interior of the glossa. The intermediate region bears the paraglossæ, the basal halves of which merge with the glossa to form a single body; the cavity of either paraglossa also becomes confluent with that of the glossa (Plate **4**, Fig. 29). The

lateral surfaces of the glossa are strongly concave (Plate **4**, Fig. 29), and receive the maxillæ, which lie adjacent to it. The maxillæ normally approach each other in the space left free between the upper surface of the end of the glossa and the under surfaces of the paraglossæ (Plate **1**, Fig. 3). The glossa is prolonged behind into a pair of slender, diverging, chitinous stalks or legs, by von Stummer-Traunfels termed the "Stützgerüst" (Figs. 10, 22, 23, *pd.'*). The enlarged base of each bears some resemblance to a human foot (*pd.*). The toe of the foot, which underlies the cardo of the maxilla, is attenuated into a long ligament which bends around the base of the cardo and attaches itself upon the outside of the base of the stipes (Fig. 10, *lig.*). The dorsal edge of the stalk is extended and modified to form a second ligament (*lig.'*), which, as previously stated, unites with a ligament from the toe of the cardo, a suture remaining to indicate where the two ligaments united.

The glossa is lined with a layer of well developed pigmented epithelium, containing large oval nuclei (Plate **1**, Fig. 3). A central tubular cavity is left, however, which is a branch of the general body cavity. The possible glandular nature of the tongue will be discussed later.

Labium.

The labium, or lower lip (Plate **3**, Fig. 24), is a single plate, formed by the union of two lateral plates, as indicated by a conspicuous median suture. Either half of the labium is divided by sutures into three distinct regions. The anterior region (*plp.*) bears five prominent tubercles, from the apex and sides of which project several subulate bristles. The intermediate region (*men.*) is of lunate form, thickly chitinized along the mesal margin, hinged along the posterior curving suture, and bears a few setæ. The posterior and most extensive region (*sb'men.*) is almost divided into two sclerites by an oblique suture, which however becomes obsolete at its posterior end. This third region is laterally fixed to the clypeus, is posteriorly distinguished from the gula by a deep oblique suture, and bears a number of setæ, many of which are barbellate. The halves of the labium are firmly fused together, except anteriorly, and are not easily separable; but the fusion involves only the ventral margins of the mesal faces of both plates, in such a way that a trough or gutter is left within the buccal cavity (Fig. 25, and Plate **4**, Figs. 28, 29, *sul.*). At the posterior end of the median longitudinal suture begins the *linea ventralis* of Tullberg (Fig. 24, *ln. v.*), an external median ventral gutter formed by two thin longitudinal ridges of chitinous cu-

ticula, the margins of which approach each other without uniting, as is shown by transverse sections (Figs. 26 and 27). This linea ventralis may be traced back along the median ventral line of the body as far as the lobes of the ventral tube, which is a median appendage of the first abdominal segment; between the body segments, however, the linea ventralis suffers considerable interruption. I shall return to this peculiar structure when considering the glands of the head.

The morphology of the labium of Collembola has never been elucidated, and is difficult to understand, even after careful comparison with other orders of insects. I am at present able to offer only a few suggestions upon the subject. The glossa and paraglossæ have no connection with the labium, although fused with each other. Labial palpi have been regarded as absent by other authors, yet the anterior regions of the labium, which are doubtless tactile in function, may perhaps be palpi in a morphological sense also. If a maxillary palpus of this same insect with its setigerous tubercles be imagined as having become sessile, through a shortening of the stalk, we have a counterpart of the terminal lobe of the labium. I see no serious objection to considering the two remaining sclerites of either side as mentum and submentum, although the presence of a nearly obsolete suture, tending to divide each submentum into two sclerites, certainly complicates the matter.

The movements of the labium are effected by five pairs of muscles, of which three are elevators and two depressors. I shall describe these muscles as they appear in successive sagittal sections, beginning at the median plane.

1. *Posterior depressor.* This is the shortest of the labial muscles, and is inserted on the ventral wall close to the median plane (Plate **1**, Fig. 3, and Plate **3**, Fig. 25, *1. dep. p.*). This muscle arises from the dorsal surface of the thick chitinous wall of the salivary duct and runs forward, downward, and inward to its insertion. It opens the mouth by pulling upon the under surface of the lower lip. All the lateral muscles are seen tranversely sectioned in Plate **4**, Figures 28–31.

2. *Mesal elevator.* This muscle runs along the upper and inner margin of each half of the labium (Figs. 3 and 25, *2. lvt. ms.*). The insertion is at the anterior extremity, and the origin close beside that of the preceding muscle, on the same chitinous duct. The function is to close the mouth by diminishing the extent of the inner surface of the labium.

3. *Anterior depressor* (Fig. 3, *3. dep. a.*). In origin, course, and function this muscle is similar to the posterior depressor; it is more lateral, however, and is inserted somewhat in advance of its companion.

4. *Middle elevator.* This muscle (Figs. 3 and 25, *4. lvt. m.*), which is longer than any of the preceding, arises from the posterior arm of the tentorium, bends downward, follows the inner wall of the labium directly forward, and terminates at the distal extremity of the labium. The function of the muscle is like that of the mesal elevator.

5. *Lateral elevator.* This is the longest and most lateral of the labial muscles (Fig. 25, *5. lvt. l.*). As to insertion and course it is similar to the middle elevator, but its origin is more posterior and dorsal, being upon the dorsal chitinous surface of the salivary duct. The principal difference between the three elevators described is that, by reason of their different insertions, they may raise different portions of each terminal movable lobe of the labium.

The labium is supplied by the third pair of nerves from the infra-œsophageal ganglion; the nerves originate a little to one side of the median plane from the ventral part of the ganglion, and pass forward, downward, and outward into the dorsal portion of either half of the lower lip. The labium is lined with a single layer of confluent, pigmented, hypodermal cells, with moderately large, round nuclei. Beneath the bristles of either half of the labium is a cluster of large oval nuclei, which belong to filiform cells exactly like those of the labrum; ganglionic cells may also be distinguished, which bear the same relations as those described for the upper lip.

The Cephalic Glands.

The glands in the head of Orchesella comprise two pairs, the principal pair lying in the base of the head and occupying most of the region behind the maxillæ (Plate **1**, Fig. 2, *gl.*). Each of the larger glands consists of a single tube, which is thrown into several longitudinal convolutions. The deeper half of the tube is of larger caliber than the remainder, and constitutes the secretory part (Plate **4**, Fig. 32, *gl.*), while the more superficial half is distinctly smaller in caliber and simply conductive in function (Fig. 32, *dt.*). The chitinous evacuating duct of either side finally runs forward and downward on the mesal side of the gland, passing just beneath the maxillar evagination and trending toward the median plane. The ducts open separately on either side of the extreme proximal portion of the labial cleft (Plate **3**, Fig. 25, *dt.*). As the mesal surfaces of the two halves of the labium are fused together along their ventral edges only, a trough or gutter (*sul.*) is left above the line of fusion, through which the secretion may run forward to the mouth opening.

The epithelium of the glandular portion consists of large polygonal cells (Plate **4**, Fig. 33), containing large oval nuclei. The granular cytoplasm is confined to the base of each cell, where it forms a dense, deeply staining mass; the portion of the cell adjacent to the lumen of the gland contains a clear non-staining substance, which sometimes appears to occupy large vacuoles and is probably secreted fluid. A delicate chitinous intima may be distinguished as well as a thin basement membrane. The dorsal wall of the gland proper is connected to the ventral wall of the maxillary pocket by columnar cells (Fig. 33) with small oval nuclei and fibrous cell body. These are probably modified hypodermis cells; between them may be seen unmodified cells, which are flattened against the cuticula.

Transverse sections show the evacuating duct to be chitinous throughout its whole length (Fig. 34), the component cells being indicated by small, round nuclei only. The basement membrane is thin, but the chitinous intima is thick.

In my account of the anatomy and function of the principal cephalic glands, I differ widely from Fernald ('90) and also from Willem et Sabbe ('97). According to Fernald ('90, p. 63), a curious anatomical relation exists between the "salivary glands" and the ventral tube or "vesicle" of Anurida maritima. I give the author's own words: "Passing forward [from the cleft of the abdominal vesicle] on the ventral median line of the body to a median cleft in the lower lip is a small tube, in the formation of which both hypodermis and cuticula take part. In the posterior portion of the head are a pair of glands which resemble salivary glands and which I regard as their homologues here. From these glands a duct leads forward and soon fuses with its fellow, and the median duct thus formed passes along the under surface of the buccal cavity to a median cleft of the lower lip, where, instead of emptying into the mouth, it turns downward and joins the ventral tube just described. This remarkable relation of the parts concerned I am unable to explain, although sure that no error of observation was made."

Willem et Sabbe ('97, pp. 131, 132) have recently offered an explanation of the peculiar relation which Fernald described but left without an interpretation; they claim that the ventral tube of Smynthurus is an adhesive organ, covered with a glutinous substance and add: "Ce liquide est sécrété, comme l'a annoncé Fernald pour Anurida maritima, par deux glandes situées dans la tête, chez Sminthurus fuscus, elles sont logées dans la région postérieure de la cavité céphalique et occupent les protubérances verticales postérieures de la tête. Chacune d'elles est constituée

par un tube contourné qui, dans sa région glandulaire, se composé de cellules plates à gros noyaux et à bordure striée; le conduit excréteur, plus étroit, débouche à l'extrémité postérieure de la fente médiane de la lèvre inférieure. Ces glandés, découvertes chez Macrotoma flavescens par Tullberg, qui les considérait comme glandes salivaires, ont été figurées ensuite chez *Lipura* [Aphorura] ambulans par Nassonow qui les fait se déverser dans la cavité buccale; elles ont été signalées chez Anurida maritima par Fernald, qui a découvert leur véritable rôle. De l'orifice de la glande jusqu'au tube ventral, la sécrétion suit une gouttière chitineuse incomplète, qui court sur la ligne médiane de la face inférieure de la tête et du thorax, en passant entre les pattes; elle descend le long du tube ventral pour aboutir au sillon qui sépare les deux lobes de cet organe."

I do not deny these observations upon Anurida and Smynthurus, especially in the face of Fernald's positive assertion, — I have not examined those genera with reference to this question, as to do so would take me beyond the scope of this paper, — but I have been unable to confirm the observations in the case of Orchesella. In this species there is no common median duct, although the approximating sides of the labial cleft might possibly be mistaken for such. I find no distinct opening through the labium into the *linea ventralis;* this structure is, as I have described, and as Willem et Sabbe admit, "une gouttière chitineuse incomplète," being always more or less open (Plate 3, Figs. 26, 27) throughout its length, and moreover becoming more or less interrupted between the body segments. In short, it is doubtful if it can have the function of conveying even a viscid fluid. Furthermore, the exsertile processes of the ventral tube are themselves well provided with unicellular glands already described by Sommer ('85), but disregarded by Willem et Sabbe ('97), sufficient to furnish the viscid secretions. It is my opinion, then, that the larger cephalic glands of Orchesella are truly salivary glands, as are those of Macrotoma [Tomocerus] and Lipura [Aphorura] in the opinion of Tullberg and Nassonow.

The second pair of glands lie close to the skull on either side of the head, between the bases of the mandible and maxilla. Each gland consists of a somewhat conical mass of secreting cells converging downward to a chitinous duct which follows the skull down, between the mandible and maxilla, becoming triangular in cross section, and opens through the lateral wall of the mandibular pocket, about half way down the mandible.

The glandular cells (Plate 4, Fig. 35, *gl.*) are polygonal, with large

oval nuclei; the cytoplasm forms a close network, the interspaces of which contain a clear substance.

The transition is abrupt between the gland and the duct, which ends blindly (Fig. 35, *dt.*). The end of the duct, however, is provided with many pores, which facilitate the passage of secreted fluid through the thick, chitinous wall of the duct. The mouth of the duct is distinctly marked by ectodermal pigment, which is reflected from the wall of the buccal cavity and lines the lumen of the duct for a considerable distance (Fig. 31, *lu.*).

The pivot of the mandible (*cdx.*), resting in its stirrup, abuts against the gland, as shown in Figure 35. This leads me to think that the lubrication of the pivot may possibly be an incidental function of the gland.

This pair of glands evidently corresponds to a pair described for Smynthurus by Willem et Sabbe ('97, p. 132), who state, however, that "Les conduits excréteurs des différentes cellules d'un même côté se réunissent en un canal collecteur qui, de la base de la mandibule, descend obliquement pour se terminer dans la partie supérieure de la cavité buccale, dans l'angle formé par la mandibule et l'hypopharynx." In Orchesella, the gland cannot be said to open in the same place.

Regarding the possibility of the existence of a lingual gland, I may say that the base of the glossa is lined with epithelial cells which are unusually large and contain large oval nuclei (Plate 1, Fig. 3). There is apparently a median opening, or at least a very thin place on the upper wall of the glossa (Plate 3, Fig. 23, and Plate 4, Fig. 28, *of.*), but I have not been able to trace its relation to underlying tissues on account of the extreme delicacy and brittleness of the glossa, resulting in unavoidable distortions in the process of sectioning. On the other hand, the central cavity of the glossa is undoubtedly a part of the general body cavity, so that the evidence in favor of the tongue being glandular is at most very slight.

The Physiology of the Mouth-Parts.

Almost complete ignorance of the physiology of the mouth-parts has been but the natural consequence of an incomplete knowledge of their anatomy. Obviously, very little can be learned by direct observation or experiment; much may be inferred, however, from the structure and relations of the organs. I have already described the action of the muscles which are concerned, and may now briefly trace the history of the food until the stomach is reached.

Orchesella cincta is a common species among decomposing leaves and in moss; it is most abundant among decaying pine needles and twigs, upon which it feeds. The stomach usually contains minute irregular fragments of wood, and the insect thrives when confined in a glass tube with a moistened piece of decaying pine wood.

The observing Dr. Fitch ('63) is the only naturalist who has given any account of the feeding habits of Collembola. I quote his observations ('63, p. 672) upon Smynthurus hortensis: "These Garden Fleas are so minute that the human eye without the aid of glasses is wholly unable to inspect their movements. The following observations will therefore be the more interesting to the reader. It is some years since that I noticed several of these insects on a piece of new pine board lying in the garden. Wondering what they could find to attract them to that situation, where I thought the odor of any turpentine in the wood would rather make it repulsive to them, I was able to observe their operations by approaching a magnifying glass to them gently, so as not to alarm them and cause them to skip away, — the light colored surface of the new wood enabling me to inspect their movements much more accurately than could be done were they standing upon a darker colored ground. Several of them were noticed, here and there, to have grasped in their mouths what appeared to be an exceedingly minute flexible fibre of the wood, fine as a fragment of a spider's web; and they were pulling backward, at the same time shaking their heads slightly, evidently to tear off these fibres. One of the fore legs was frequently used to crowd this fibre more and more into the mouth, whenever it became peeled up and too long to pull upon to advantage. Everything indicated that it was for the purpose of food that they were thus tearing off this fine fuzz from the surface of the new board. At one place was a small black spot in the board, caused apparently by some old disease in the wood at this point, which rendered it more soft and palatable to the insects, for two of them were here busily occupied in gnawing the particles of matter from the surface, as it seemed."

The stout setæ which project from the labrum, palpi, and labium, and surround the mouth, are probably tactile in nature. It is possible that the food is moistened with saliva before being taken into the mouth, as the median trough of the labium is well adapted to convey saliva to the border of the mouth.

In order to seize food, the mandibles leave their sockets and are protruded a little from the mouth. The tips of the mandibles, by lateral movements, grasp fibres of decaying wood, which are held between the

terminal incisive teeth, the four teeth of the left mandible interlocking with the five of the right one. The food is pulled into the mouth by the retraction of the mandibles, assisted by the upper and lower lips, then meets the secretions poured into the buccal cavity by the two pairs of glands, and is grasped by the claws of the terminal maxillary lobes, which move laterally; the entire maxilla may also perform lateral movements. The maxillæ are situated on either side of the tongue, and their tips interlock in the space left between the glossa and paraglossæ, so that the retraction of the maxillæ — which is slight, however, as contrasted with that of the mandibles — must pull the food along the dorsal surface of the glossa, and through the space which intervenes between the paraglossæ. In this operation, the laciniæ brush along the surface of the glossa, which is curved so as to conduct the food between the paraglossæ. On the concave dorsal surfaces of the paraglossæ the food meets the grinding faces of the mandibles, the upward rotary movement of which may carry it to the projecting teeth of the paraglossæ. I have sometimes found particles of wood held between the teeth of the paraglossæ and those of the epipharynx, which is opposite. The coarse ventral teeth of the mandibles crush the woody fibres preparatory to a finer comminution by the denticulated molar surfaces. In the grinding process, the powerful adductors play the principal part, supplemented by rotary movements and possibly also by forward and backward rubbings. The downward rotary movement is much stronger than the reverse, judging from the size and number of the muscles concerned in the two acts. During mastication the mandibles are probably withdrawn into their chitinous sockets, where the pivots encounter firm resistance. The pivots are perhaps lubricated by glands already described.

The comminuted food of the pharynx is sucked into the œsophagus. This occurs by the constriction and subsequent dilatation of the fore gut. Once within the œsophagus, the food may be forced back by peristaltic action, resulting from the successive contraction of constricting muscles, until the stomach is reached, where a valve prevents the return of the food into the gullet.

Although Collembola are classed as mandibulate insects, it is evident that they are also suctorial. The Collembola are closely related to Campodea, a generalized type which is regarded as the representative of a primitive form from which more specialized insects have been derived. As already suggested by Lubbock, we may imagine the primitive insect to have possessed mouth-parts resembling those I have described, ca-

pable of further modification in either the mandibulate or suctorial direction. Indeed, the latter modification has already occurred in the Collembolan genus Neanura.

In conclusion, I desire to thank Mr. Samuel Henshaw, whose knowledge of entomological literature has been of great service to me. I wish to acknowledge my special indebtedness to Professor Mark; for his careful and kind supervision have been my greatest aid and encouragement.

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EXPLANATION OF PLATES.

[All figures are of Orchesella cincta L.]

ABBREVIATIONS.

NOTE. — Each muscle is numbered in agreement with the text.

a.	Anterior.
abd.	Abductor.
add.	Adductor.
at.	Antenna.
atc.	Articulation.
bac.	Rod.
br.	Arm.
br. a.	Anterior arm.
calc.	Shoe.
car.	Cardo.
cav. buc.	Buccal cavity.
cdx.	Pivot.
cht.	Chitinous.
cl. gn.	Ganglion cell.
clyp.	Clypeus.
cpt.	Head.
cra.	Cranium.
crs. ba.	Basal ridge.
c'stt.	Constrictor.
cta.	Cuticula.
cx.	Hcel.
d.	Dorsal.
de.	Teeth.
dep.	Depressor.
dil.	Dilator.
dt.	Duct.
e'phy.	Epipharynx.
e'th.	Epithelium.
exp.	Expansion.
f.	Frontal.
fac.	Surface.
ga.	Galea ?
gl.	Gland.
gls.	Glossa.
gn. inf'æ.	Infra-œsophageal ganglion.
gn. su'æ.	Supra-œsophageal ganglion.
gu.	Gula.
h'drm.	Hypodermis.
i.	Intima.
i'cis.	Incisive.
l.	Lateral.
la.	Plate.
lab.	Labium.
lbr.	Labrum.
lcn.	Lacinia.
lig.	Ligament.
ln. v.	Linea ventralis.
lu.	Lumen.
lvt.	Elevator.
m.	Middle, median.
m....m.	Median line.
mb. ba.	Basement membrane.
md.	Mandible.
men.	Mentum ?
mol.	Molar.
ms.	Mesal.
mu.	Muscle.
mx.	Maxilla.
n.	Nerve.
nat.	Natural size.
nl.	Nucleus.
ocl.	Ocellus.
œ.	Œsophagus.
of.	Orifice.
or.	Mouth.
p.	Posterior.
pa'gls.	Paraglossa.
pd.	Foot.
pd'.	Footstalk.
phy.	Pharynx.
pig.	Pigment.
pi'my.	Perimysium.
pli.	Fold.
plp.	Palpus.
pr'j.	Projection.
pr'j. con.	Conical projection.
pr't.	Protrusor.
ret.	Retractor.
rot.	Rotator.
sb'men.	Submentum ?
set. sns.	Sensory bristle.
sta.	Stirrup.
stp.	Stipes.
sul.	Trough.
sut.	Suture.
tnd.	Tendon.
tnt.	Tentorium.
v.	Ventral.

PLATE 1.

Fig. 1. External aspect of the mouth. × 116.

Fig. 2. Diagram of the head, seen from the right side, to show the relations between certain organs. × 55. The small figure in a circle represents the natural size of the head.

Fig. 3. Reconstruction from sagittal and parasagittal sections of the left half of the head, imagined as seen from the right side. The numbers 28, 29, 30, and 31 refer to figures of Plate **4**, bearing corresponding numbers, which represent transverse sections made in the positions of the numbered lines of this figure. × 220.

Fig. 4. Transverse section of the œsophagus. × 530.

Fig. 5. The tentorium, viewed from the right side; reconstructed from serial sagittal sections. × 220.

Fig. 6. Dorsal aspect of the tentorium; reconstructed from frontal sections. × 220.

Fig. 7. Sagittal section passing through the tentorium and adjacent structures. × 440.

Fig. 8. Parasagittal section of the labrum. × 220.

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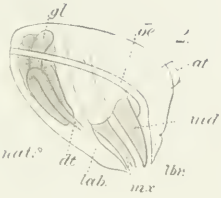
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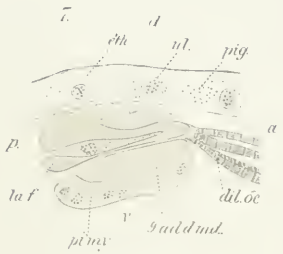
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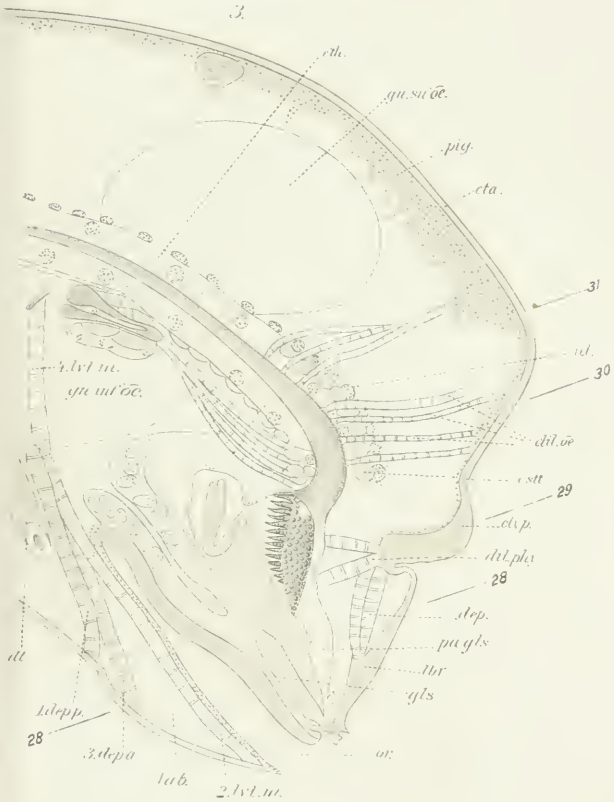
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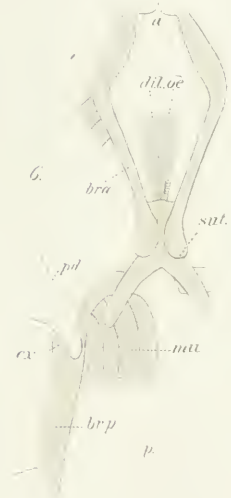
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PLATE 2.

Fig. 9. Internal aspect of the labrum and clypeus, to show the epipharynx. × 220.

Fig. 10. Dorsal aspect of the internal mouth-parts, *in situ;* the paraglossæ are omitted. From dissections and potash preparations. × 116.

Fig. 11. Ventral aspect of the apex of the right mandible. × 440.

Fig. 12. Ventral aspect of the molar region of the right mandible. × 440.

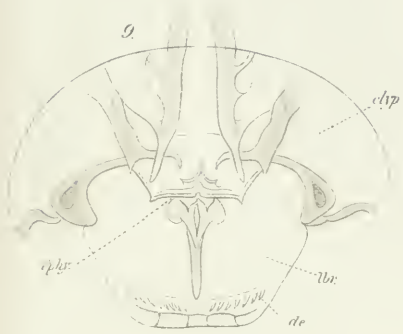
Fig. 13. Dorsal aspect of the base of the left mandible, *in situ;* from a dissection. × 440.

Fig. 14. Dorsal aspect of the left mandible with its muscles. Reconstructed from serial sections, aided by dissections. × 116.

Fig. 15. Ventral aspect of the right mandible, to show certain muscles, and to supplement Fig. 14. From a dissection. × 116.

Fig. 16. Posterior aspect of a transverse section of the right mandible, cut near the anterior angle of the triangular orifice. × 220.

Fig. 17. Three ganglion cells from within a mandible. × 530.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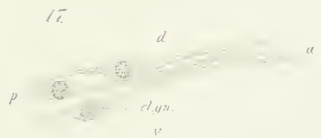
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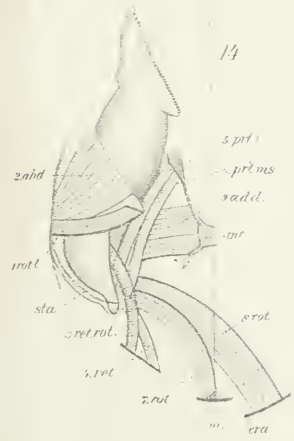
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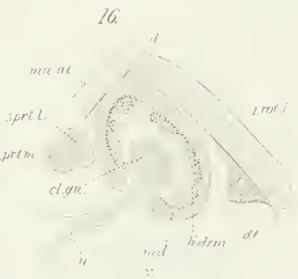
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PLATE 3.

Fig. 18. Dorsal aspect of the framework of the left maxilla; from a dissection. × 116.

Fig. 19. Dorsal aspect of the head of the right maxilla; from a dissection. × 530.

Fig. 20. Dorsal aspect of the left maxilla, with most of its muscles. Reconstructed from serial sections, aided by dissections. × 116.

Fig. 21. Dorsal aspect of the more ventral portion of the left maxilla; supplementary to Fig. 20. From serial sections and from dissections. × 116.

Fig. 22. Dorsal aspect of the right paraglossa, with underlying glossa; from dissections. × 440.

Fig. 23. Dorsal aspect of the glossa; from dissections. × 440.

Fig. 24. Ventral aspect of the right half of the labium; from a dissection. × 220.

Fig. 25. Dorsal view of the left half of the labium; reconstructed. × 220.

Figs. 26 and 27. Transverse sections of the *linea ventralis*. × 530.

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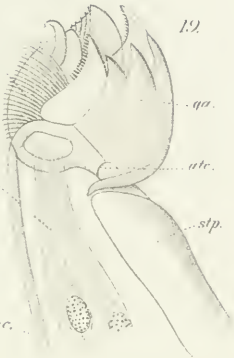
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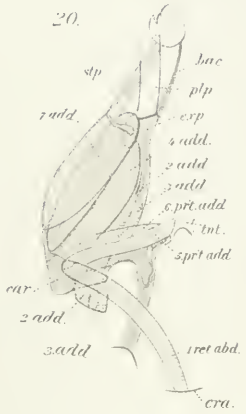
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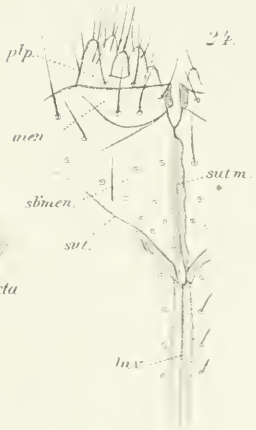
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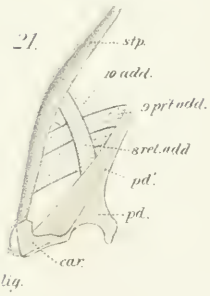
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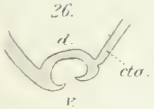
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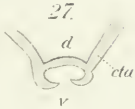
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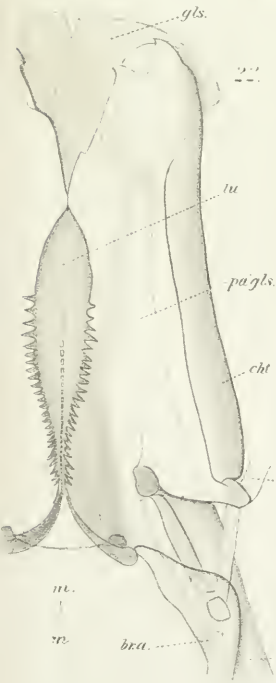
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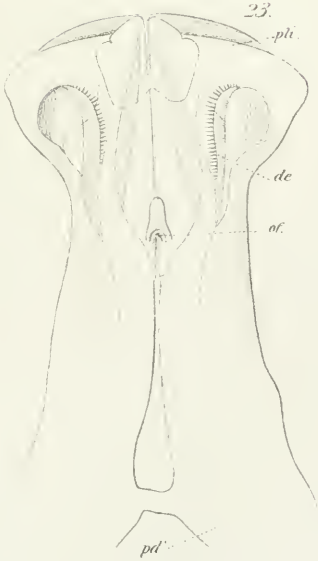
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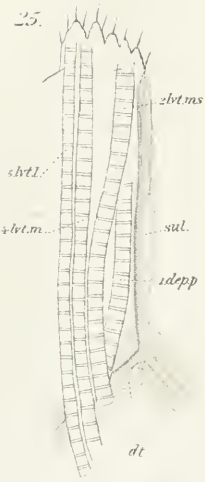
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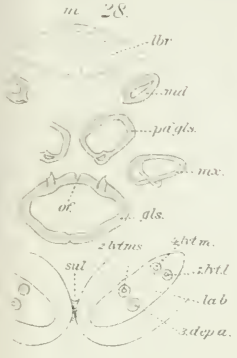
PLATE 4.

Figs. 28–32. Sections nearly transverse to the mouth-parts, numbered in sequence, beginning near the mouth, and cut approximately in directions indicated by the numbered lines of Plate **1**, Fig 3. Figs. 28–31, × 220; Fig. 32, × 116.

Fig. 33. Transverse section through a secretory region of one of the larger cephalic glands. × 440.

Fig. 34. Transverse section of an evacuating duct of the same gland. × 440.

Fig. 35. Sagittal section of one of the pair of smaller cephalic glands. × 220.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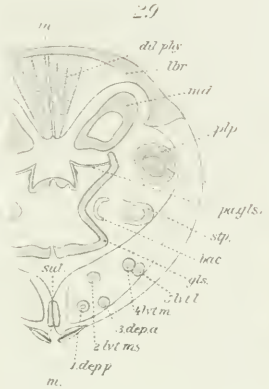
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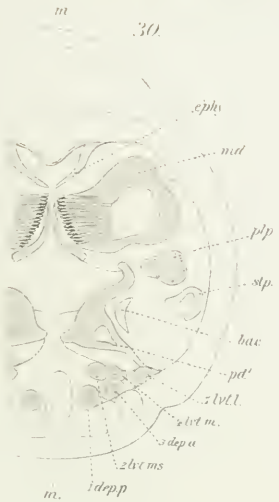
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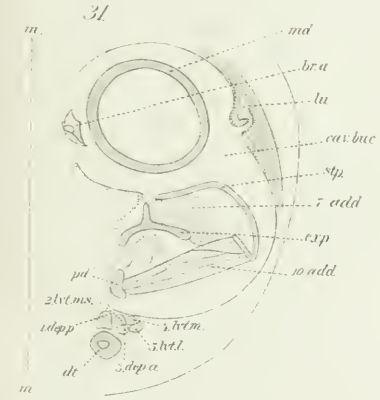
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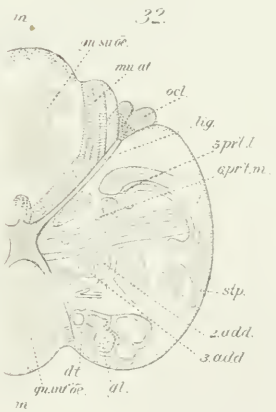
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THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos.

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[3] XV.[16] The Stalk-eyed Crustacea.

S. GARMAN. The Fishes.

W. GIESBRECHT. XVI.[15] The Copepods.

A. GOËS. III[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

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H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June, 1891, 16 pp. ; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates.

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull. M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z., Vol. XXV., No. 1, September, 1893, 25 pp.

[8] Bull. M. C. Z., Vol. XXV., No. 2, December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4, January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C. Z., Vol. XXV. No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV. No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z., Vol. XXV. No. 10, October, 1894, 109 pp., 12 Plates.

[14] Mem. M. C. Z., Vol. XVII. No. 3, October, 1894, 183 pp., 19 Plates.

[15] Bull. M. C. Z., Vol. XXV. No. 12, April, 1895, 20 pp., 4 Plates.

[16] Mem. M. C. Z., Vol. XVIII., April, 1895, 292 pp., 67 Plates, 1 Chart.

[17] Bull. M. C. Z., Vol. XXVII., No. 3, July, 1895, 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol. XXVII., No. 4, August, 1895, 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895, 14 pp., 3 Plates.

[20] Bull. M. C. Z., Vol. XXIX., No. 1, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M. C. Z., Vol. XXIII., No. 1, September, 1897, 92 pp., 15 Plates.

[22] Bull. M. C. Z., Vol. XXXI., No. 5, December, 1897, 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol. XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M. C. Z., Vol. XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, July 1899, 4 pp., 1 Plate.

PUBLICATIONS

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STUDIES FROM THE NEWPORT MARINE LABORATORY.
COMMUNICATED BY ALEXANDER AGASSIZ.

XLII.

LONGITUDINAL FISSION IN METRIDIUM MARGINATUM MILNE-EDWARDS.

By G. H. Parker.

With Three Plates.

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XLII.

Longitudinal Fission in Metridium marginatum Milne-Edwards.

By G. H. Parker.

From early times double specimens of different species of Metridium have been observed and recorded. Thus in the last century Dicquemare ('75, p. 229, Tab. VI. Fig. 2) described and figured a specimen of M. dianthus with two complete oral disks. Similar specimens were observed by Johnston ('47, p. 233), who called them monstrosities, and interpreted them as cases of coalescence brought about by the gregarious habit of the species. Thorell ('59, p. 10) and Gosse ('60, p. 20) likewise mentioned specimens with two disks as monstrosities, though Gosse also expressed the opinion that they were due to a tendency to spontaneous division. Foot ('63, p. 64), who confirmed the observations of his predecessors on the occurrence of animals with two oral disks, also recorded the discovery of a specimen with two mouths on one disk and further stated that these aberrations from the normal form are merely to be considered as monstrosities. G. Y. and A. F. Dixon ('91, p. 20), in reporting the occurrence of specimens with two disks as well as those with two mouths on one disk, mention them likewise as monstrosities. Carlgren ('93, p. 109), after stating his belief that longitudinal division is not uncommon in M. dianthus, adds : "Mehrere Forscher scheinen Formen mit zwei Mundscheiben als monströse ansehen zu wollen. Es kann doch wohl als eine begonnene Längsteilung, die nicht zu Ende geführt ist, betrachtet werden."

Double specimens of M. marginatum were observed as early as 1847 by Professor Louis Agassiz, among whose unpublished drawings are three figures, two of which (Plate I. Figs. 2, 3) bear this date and probably represent two views of the same animal ; the third figure (Fig. 1) bears the date of 1860. These figures were unaccompanied by any notes bearing

on the question of fission, but in the "Seaside Studies," published in 1865 by Elizabeth C. Agassiz and Alexander Agassiz ('65, p. 11), longitudinal fission is stated to occur in this species.

Finally, Torrey ('98, p. 347), who studied the Californian species M. fimbriatum, described double specimens which he believed to be in process of fission.

The material upon which the present paper is based consisted of ten specimens of Metridium marginatum, collected in part by myself and in part by others. The sources of this material are acknowledged in the account of the specimens given with the description of the figures. Here each specimen has received a distinguishing letter. To the gentlemen whose names are mentioned as having obtained certain specimens for me, I wish to express my indebtedness. I am also indebted to Dr. H. C. Bumpus, Director of the Laboratory at the United States Fish Commission Station at Wood's Hole for many courtesies shown me while working at the station, and I am under special obligations to Mr. Alexander Agassiz for the privilege of working at the Newport Laboratory, and for the use of unpublished drawings made for Professor Louis Agassiz.

The animals collected were for the most part stupefied by means of magnesium sulphate in sea water, Tullberg's well known method, and subsequently hardened in chromic acid and dissected by hand.

The specimens naturally fall into two groups: first, those with two mouths on one disk, of which there were two examples, specimens A and B (Plate II. Figs. 6 and 7); and secondly, those with two complete oral disks, of which there were eight, C to J (Plate II. Figs. 4 and 5, Plate III. Figs. 8–14).[1] The two specimens (Plate I. Figs. 1–3) figured by Professor Agassiz belong also to this group. These two groups were not only distinguished by external anatomical differences, but also by certain internal characteristics. In both representatives of the first group the œsophageal tubes were Y-shaped, the single inner end opening into the gastrovascular cavity and the two outer ends opening each through a mouth. In specimen A (Plate II. Fig. 6) the bifurcation was close to the oral disk and the œsophagus was for the greater part of its extent a single flattened tube. In specimen B (Fig. 7) the œsophagus was double excepting at its extreme inner end, so that its form is perhaps more correctly described as Y-shaped. In all the specimens in the second group the œsophageal tubes were entirely distinct from their super-

[1] One of these eight, specimen J, was sacrificed in an attempt to rear it; hence I have been able to study the internal anatomy of only seven such specimens.

ficial to their deep ends (Plate III. Figs. 8–14). In the material at hand, then, completely distinct disks were always associated with completely separate œsophageal tubes and single disks bearing two mouths with only partially separate tubes.

Although the œsophageal tubes have been described as though they were either entirely distinct or united only through their own substance, in two of the nine animals studied there were special membranes attaching one tube to the other. In specimen C (Plate III. Fig. 8) the two tubes were held together by a single membrane which extended from their oral to their pedal ends, but which failed of connection with the inner surfaces of both oral and pedal disks. In specimen B (Plate II. Fig. 7) two such membranes were present and extended from the region where the œsophageal tubes united almost to the oral disk. Both membranes were closely attached to the tubes, and were deficient only next the oral disk; consequently the cavity which they enclosed was entirely cut off from the gastrovascular cavity except near the oral disk, where it opened freely over both membranes. Although the membranes in these two specimens had the general appearance of mesenteries, they lacked the characteristic muscle bands and mesenteric filaments. Membranes much like these, but in one case bearing mesenteric filaments, have been observed in M. fimbriatum by Torrey ('98, p. 347).

In all specimens excepting B, F, and H, each mouth was monoglyphic; in B (Plate II. Fig. 7) and F (Plate III. Fig. 11), each animal had a monoglyphic and a diglyphic mouth; in H (Plate III. Fig. 13) one mouth was monoglyphic and the other aglyphic. In those cases where the œsophageal tubes were united, A and B, the siphonoglyphs were, notwithstanding this union, distinct throughout their whole lengths. The siphonoglyphs of any double animal apparently do not occupy random places, but are usually arranged symmetrically with reference to the assumed plane of division. This is easily seen in Figures 8, 10, 12, and 14 (Plate III.). Its significance in connection with fission has been pointed out by Torrey ('98).

Of the complete mesenteries the directives were always in pairs, and always attached to the siphonoglyphs. Their arrangement consequently corresponded to that of the siphonoglyphs, and hence need not be described. The non-directives were also always in pairs, though not infrequently one member of such a pair was incomplete. This occurred in eleven cases in a total of 107, or in about 10% of the mesenteries in the specimens examined. Including under the head of pairs of complete mesenteries those cases in which only one member of a pair is complete,

the number and general distribution of the non-directives is indicated in the following tabulation.[1]

Specimen	A	B	C	D	E	F	G	H	I	
Left Directive Pair	1	1	1	1	1	1	1	1	1	
Intervening Non-directive Pairs	1	2	3	2	1	2	3	16	3	= 33
Right Directive Pair	1	1	1	1	1	1	1	0	1	
Non-directive Pairs	8	2	16	7	8	3	13	0	5	= 62
Additional Directive Pair		1				1				
Non-directive Pairs		6				6				= 12
Total Non-directive Pairs										107

The numbers of non-directives in the animals with two mouths bear an important relation to those of animals with single mouths. In an enumeration of the mesenteries in the latter, made some time ago (Parker, '97), I found that in 131 specimens the pairs of non-directives were never fewer than three and never more than fourteen, and that the average was about five and a half pairs (5.6+) for each individual. In the nine double-mouthed specimens the non-directives were never fewer than eight pairs, nor more than nineteen, and the average was a little less than twelve (11.9—) for each individual. It thus appears that the double-mouthed specimens have almost exactly twice as many non-directive mesenteries as the single-mouthed ones.

As can be seen from the tabulation already given, as well as from the figures, the non-directives are unevenly but characteristically distributed, there being usually a large group on one side and a small group on the other side of the pairs of directives, called rights and lefts. Even in specimens B, F, and H (Figs. 7, 11, and 13), where the directives are more or fewer than two pairs, this same peculiarity in distribution may be said to exist. In another respect the distribution of the non-directives is remarkably regular. In specimen I (Plate III. Fig. 14) a pair of non-direc-

[1] In this table the term left directive pair is applied to the pairs of directives toward the left in the figures on Plate III., and to those toward the bottom in the figures on Plate II. The corresponding pairs on the other œsophageal tubes are called right directive pairs. Right and left have in this connection only this simple descriptive significance.

tives is so placed that, while they start from nearly the same place on the outer wall, they pass to different œsophageal tubes. The pair opposite these has similar connections, so that at this stage the assumed plane of division may be said to pass through primary entocœls on both sides. The same is true of G (Fig. 12), and Torrey ('98, Plate XXI. Fig. 1) figures what seems to be a similar case, though in his description (p. 347) he mentions only *one* pair of mesenteries as divided between the œsophageal tubes. Apparently there is some discrepancy here, but whether it is the description or the figure which is faulty, it is impossible to say. In the remaining specimens examined by me, the division plane always lay in primary ectocœls. It is noteworthy that in the nine cases studied none showed the plane of division passing through a primary ectocœl on one side and a primary entocœl on the other. In this respect the specimens were always perfectly symmetrical, a condition which may hold for other Actinians, as suggested by the symmetrical division of Zoanthus thomensis as described by Koch ('86, p. 33).

The incomplete mesenteries formed what were usually irregular groups in the primary ectocœls. Their arrangement seemed to bear so little on the question of division that I have not attempted a description of them. In the figures of transverse sections the incomplete mesenteries are either represented in full in a given primary ectocœl or their presence is indicated by *x*. Primary ectocœls not marked in one or other of these ways contained no incomplete mesenteries.

Of the double-mouthed specimens whose sexes could be determined, four were females and three were males. No evidence of hermaphroditism was observed, though the specimens were carefully scrutinized in this respect. Obviously the double-mouthed condition is not peculiar to either sex.

The interpretations that have been placed upon these double-mouthed specimens have already been stated. Johnston's idea that they have arisen by the fusion of two originally independent individuals seems to me entirely unwarranted. M. marginatum is represented by individuals showing extreme variations in color and markings, and yet the two members of all double specimens seen by me have been strikingly similar. If fusion were the means of forming double animals, particolored combinations ought occasionally to occur, but such I have never seen.

There remain then the two suggestions of monstrosities and of stages of fission. To test which of these was the correct interpretation, I attempted to watch the process in what might be assumed to be a dividing individual. This animal was found at Wood's Hole, August 6th, 1898,

and was kept under observation there during August and later at Beverly Bridge till October 3d of the same year, a period of some eight weeks. During this time the animal was kept in open sea water in a marked locality, not in a laboratory aquarium, which my former experience had taught me might be unfavorable. When first seen its pedal disk measured about three centimeters in diameter. It had two complete oral disks, one of which had a monoglyphic and the other a diglyphic mouth. The cleft between the two oral disks was a deep one, and I hoped soon to witness the separation of the two individuals. When about eight weeks after capture it was last seen, it had increased in size so that its pedal disk was nearly seven centimeters in diameter, but the cleft between the two parts had in no wise increased. The specimen then unfortunately disappeared from its locality. The fact that it grew considerably shows that it was under approximately normal conditions, and yet so far as fission was concerned the animal was essentially at the same stage at the end of the eight weeks as at the beginning. This coincides with the experience of Torrey ('98, p. 351), who in describing longitudinal fission in M. fimbriatum states that he has "not observed a single instance of full severance of individuals, though a number of dividing polyps have been kept in the laboratory for nine months." Thus direct evidence of actual fission is wanting.

If now we turn to the specimens which have thus far been described, it must be admitted that they can be arranged in a series passing from less to more completely divided individuals. This in itself, however, affords no more grounds for concluding that M. marginatum reproduces by longitudinal fission than a similar series of partially double mammals would establish longitudinal fission for these animals. To make the proof conclusive the final products of the process must be found.

As a rule, the larger specimens of M. marginatum are sessile animals.[1] In large specimens that had undergone division the offspring ought therefore to be found together. M. marginatum further shows great variability in its colors and markings; hence such natural pairs might thus be distinguished from their neighbors, for the two descendants would of course inherit directly the surface markings of their progenitor.

A search was made for natural pairs at Beverly Bridge, a locality

[1] According to my own observation the locomotor activity of this species becomes rapidly less as the size increases. A specimen whose diameter was about 5 mm. moved as much as 9 cm. in twenty-four hours. A second one whose average diameter was about 2 cm. never moved more than 1 cm. in a day, and a large one whose diameter was about 8 cm. showed no perceptible movement in fifteen days.

where the species was represented by thousands of individuals in easily accessible positions. The search was facilitated by the fact that often a given pile or plank was covered with individuals of almost exclusively one type of coloration; almost all in one restricted location were orange-colored, or brown, or whitish, etc. This is doubtless due chiefly to reproduction by fragmentation from the edge of the pedal disk, a non-sexual process which insures to the offspring the minute characteristics of the parent. My object was to find pairs of animals individually separate, but situated next each other and strikingly similar in color and markings, though entirely unlike those surrounding them. Such instances, if found, would afford strong evidence in favor of the actual occurrence of longitudinal fission. A search of some three hours' duration, in which between two and three thousand specimens were inspected in their attached positions, resulted in the discovery of six such natural pairs. These were pairs which fulfilled the requirements of the case in every respect, in that they were composed each of two animals strikingly similar in size and coloration, and entirely isolated from others of a like kind by a surrounding group of individuals unlike them. What seemed to be natural pairs of this kind were often met with in other places, but as it was important to accept only pairs that were well isolated, and as these would naturally not occur frequently, it is not surprising that only six such pairs were found.

The six pairs were placed each in a separate bottle and transferred to the laboratory for further study. On examination all proved to be monoglyphic except one individual, which was diglyphic. The arrangement of the complete mesenteries is indicated as follows: —

First Pair	D-2-3-4-5-6-7. D-2-3-4-5-$\frac{6}{2}$-7-8.
Second Pair	D-2-D-4-5-$\frac{6}{2}$-7. D-2-3-4-5-6-7.
Third Pair	D-2-3-4-5-6-7. D-2-3-4-5-6-7.
Fourth Pair	D-2-3-4-5-6-7-8-9. D-2-3-4-5-6.
Fifth Pair	D-2-3-4-5-6. D-2-3-$\frac{4}{2}$-5-6.
Sixth Pair	D-2-3-4-5. D-2-3-4-5-6.

The first pair were found attached to a large mussel shell, and were killed and hardened without being disturbed from their natural posi-

tions. The arrangement of their siphonoglyphs and mesenteries is shown in Figure 15 (Plate III.). So far as the anatomical peculiarities of this and the five other pairs are concerned, they might well be considered as animals that had undergone division.

A further important feature of the six pairs was that in all cases members of the same pair were of the same sex. Of the six pairs, three were female and three male. This uniformity is precisely what would be expected, if these pairs arose by longitudinal fission, but quite the reverse of what we should anticipate had they been the result of accidental juxtaposition. This evidence seems to me to favor strongly the view that longitudinal fission is a regular method of increase in M. marginatum, though it does not exclude the possibility of some of the double-mouthed forms being true monstrosities. It is, however, not my belief that the specimen which was watched by me some two months and which did not progress in division was a monstrosity. I am rather inclined to Torrey's opinion, that the process of longitudinal fission is an extremely slow one. This accords with what we know of the length of life of Actinians, some having outlived human beings.

In all the double-mouthed specimens which I have examined the two partial individuals were of about equal size, so that longitudinal fission in M. marginatum may be justly described as equal. In no case have I ever observed any evidence of distinctly unequal longitudinal fission, though one of the specimens figured by Agassiz (Plate I. Figs. 2 and 3) shows some considerable inequality. In this respect M. marginatum seems to be strikingly different from M. fimbriatum, which according to Torrey ('98) divides more usually by unequal than by equal longitudinal fission.

In longitudinal fission, obviously, certain fundamental changes must occur: new siphonoglyphs and new complete mesenteries, both directive and non-directive, must be developed. In the material which I have studied I have been able to discover no trace of the formation of new siphonoglyphs. The usual symmetrical arrangement of two of the siphonoglyphs in each double animal suggests a process of division by which one original siphonoglyph gives rise to two by longitudinal splitting, as described by Torrey ('98). This is very likely one of the initial steps in longitudinal fission, though it is not necessarily essential, as the condition of specimen H (Plate III. Fig. 13) shows where the original siphonoglyph obviously did not divide.

Of the formation of new directives I have seen absolutely nothing. The production of new complete non-directives is possibly indicated in

several cases. In specimen C a curious condition was observed, which is shown in the diagram (Plate III. Fig. 8). Two complete mesenteries start from the column wall, but unite before they reach the œsophageal wall. The cavity which they enclose may be called a primary entocœl so far as their longitudinal muscles indicate, but it contains two incomplete mesenteries which pair off with the united complete ones. The condition might be interpreted as due to the splitting of a complete mesentery and the supplementary development of incomplete ones, though this interpretation is not without objections. I am more inclined to look on the condition as purely abnormal. In another case, the mesentery marked 1 in Figure 7 (Plate II.), whose mate is incomplete, is itself complete only through a small portion of its oral length. This might be a mesentery originally incomplete, which by active growth had completed itself in the oral region. A second similar case was observed among the six natural pairs already mentioned. Beyond these meagre facts, I observed nothing suggestive of the methods by which new complete mesenteries may be formed.

In all the specimens examined by me the fission was progressing from the oral toward the pedal pole of the animal, never in the reverse direction, as described by McCrady ('59, p. 275) for Actinia cavernosa, by Carlgren ('93, p. 31) for Protanthea simplex, and as may also occur in Cereactis as described by Wilson ('89, p. 38), who however did not place this interpretation on what he saw. The direction of fission in M. marginatum corresponds to that generally found in M. fimbriatum as described by Torrey ('98).

The kind of individuals produced by equal longitudinal fission in M. marginatum can be easily inferred from the foregoing account. They are usually monoglyphic, though occasionally diglyphic, possibly on rare occasions aglyphic. A pair of directive mesenteries is regularly attached to each siphonoglyph. The animals possess the usual number of complete non-directive mesenteries. These, however, do not show the regular hexamerous arrangement often met with in this species, but are as a rule very irregularly disposed. As I have elsewhere indicated (compare Parker, '97, pp. 263, 264), M. marginatum is represented by two chief types: a diglyphic one often characterized by a strictly hexamerous arrangement of its mesenteries and a monoglyphic one in which amongst other forms three sub-types, distinguished respectively by having five, six, and seven pairs of non-directives, may be recognized. The animals produced by longitudinal fission may come under any of these heads except the regularly hexamerous diglyphic form, to which

about one fifth of the numbers of specimens ordinarily collected belong. While I can confirm Torrey's ('98, p. 357) statement that diglyphic and monoglyphic individuals may arise by the same mode of non-sexual reproduction, I believe he has gone too far when he denies any possible correlation between these structural types and the methods of reproduction. Since no mode of non-sexual reproduction has been shown to give rise to a hexamerous diglyphic specimen, and since this type is represented by about one fifth of the number of individuals in the species, it seems to me still possible that this may be the result of sexual reproduction.[1] The value of this suggestion must, however, await further investigation. Should it prove true, much of the irregularity in the arrangement of the mesenteries in Metridium would be associated with non-sexual reproduction.

Summary.

The double specimens of Metridium examined had either two mouths on one oral disk or two complete oral disks. In the former cases the œsophageal tubes were incompletely divided (Y- or V-shaped); in the latter, there were two completely distinct tubes.

The mouths of the double specimens were usually monoglyphic, sometimes diglyphic, and in one instance aglyphic. Two siphonoglyphs were usually placed symmetrically to the supposed plane of division.

A pair of directive mesenteries was always attached to each siphonoglyph. There were about twice as many non-directive mesenteries in double specimens as in single ones.

In any given case the assumed plane of division passed through either two primary ectocœls or two primary entocœls, never through a primary entocœl on one side and a primary ectocœl on the other.

The double specimens were either male or female, and showed no evidence of hermaphroditism.

They are not due to fusion.

[1] In briefly discussing this question, Torrey ('98, p. 357) has quoted me in a somewhat misleading way. It is true that I suggested that the monoglyphic and diglyphic types might have the value of varieties, and also that they might be correlated with the methods of reproduction, but this was not done in one breath, as might be inferred from Torrey's statement. I mentioned the possible interpretation as varieties on page 269 of my former paper (Parker, '97), and, after stating my disinclination to accept this interpretation, I suggested on page 270 the possibility of correlation with the methods of reproduction. Why Torrey should have combined these two suggestions as one, and why he should have used quotation marks for a piece of composition which is not mine, remain to be explained.

While some may be monstrosities, the occurrence of natural pairs shows that longitudinal fission takes place.

This process is probably extremely slow.

While monoglyphic and irregular diglyphic specimens may be produced by this and other non-sexual processes, the production of regular hexamerous diglyphic specimens by non-sexual methods has not been observed. Such specimens number about one fifth of all collected, and may be the products of sexual reproduction.

CAMBRIDGE, January 13, 1899.

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EXPLANATION OF FIGURES.

All figures were taken from specimens of Metridium marginatum Milne-Edwards. In the figures of transverse sections the dotted lines represent the axes of the mouths projected upon the plane of section. All complete mesenteries are drawn. The incomplete mesenteries usually form more or less irregular groups in the primary ectocœls. Each primary ectocœl containing incomplete mesenteries either has these mesenteries represented in it or is marked with x; any primary ectocœl not designated in one or other of these ways contained no incomplete mesenteries.

PLATE I.

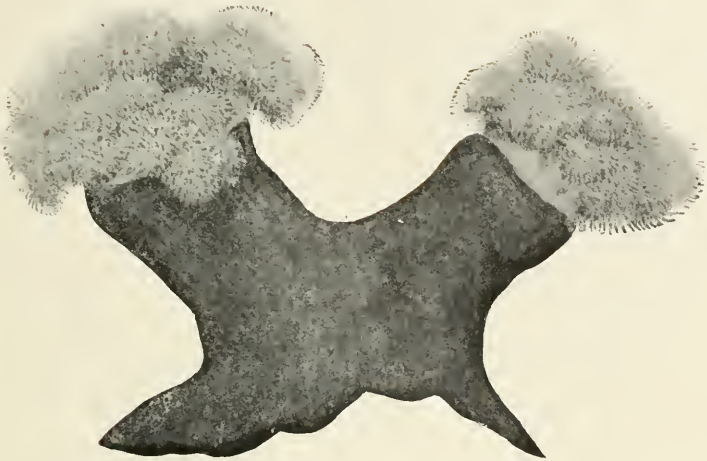
Reproductions of figures of double specimens from the unpublished drawings of Professor Louis Agassiz.

Fig. 1. Side view of a specimen with two oral disks. The original drawing is marked, "Alive in Aquarial Gardens, May, 1860." Drawn by J. Burkhardt.

Fig. 2. Oral view of a partly contracted specimen with two oral disks. The two disks show a greater difference in size than is usual with this species.

Fig. 3. Oral view of what is probably the same animal as that shown in Figure 2, but in a more contracted state.

The original drawings from which Figures 2 and 3 were taken were on the same sheet of paper, which was marked, "Boston, Sept. 28, '47." Drawn by W. H. Tappan.



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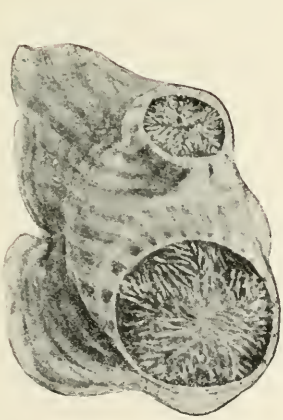

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Boston Eng. Co.

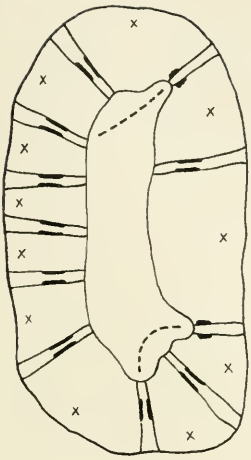
PLATE II.

Fig. 4. Lateral view of specimen D, with two complete oral disks, collected by the writer and S. F. Williams at Beverly Bridge, Mass. The arrangement of the internal parts of this specimen as seen in transverse section is shown in Figure 9 (Plate III.). Female. Natural size.

Fig. 5. Oral view of the same specimen as that shown in Figure 4. Slightly enlarged.

Fig. 6. Transverse section of specimen A, with one oral disk and two mouths. The section is taken at a level at which the œsophagus is single. Orally from this the œsophagus becomes double, each tube leading to a mouth opening. Sex not determined. Collected by B. H. Van Vleck at Cape Ann, Mass. ×2.

Fig. 7. Transverse section of specimen B, with one oral disk and two mouths. The cavities of the two œsophageal tubes were entirely distinct except near the extreme aboral end, where the two tubes united to form a single œsophagus. The œsophageal tubes are connected with each other by a pair of membranes which resemble mesenteries without differentiated muscle bands. These membranes extend from the region in which the two œsophageal tubes separate almost to the oral disk. The cavity between them opens into the gastrovascular cavity orally, since both membranes are deficient in that region. Mesentery 1, though complete orally, is incomplete through more than half of its pedal length. Collected by the writer at Wood's Hole, Mass. Female. ×3.



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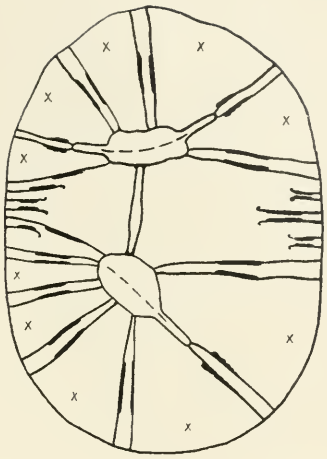

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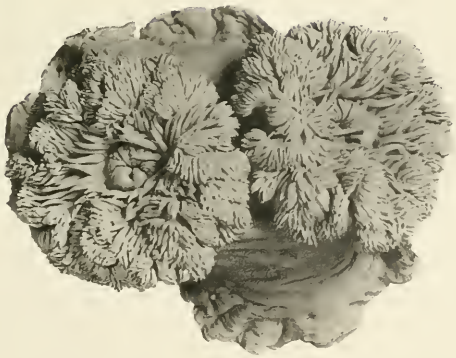

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PLATE III.

All the specimens shown on this plate, except those represented in Figure 15, had each two complete oral disks and two distinct œsophageal tubes.

Fig. 8. Transverse section of specimen C, in which the two œsophageal tubes are connected by a single membrane which reached neither the oral nor the pedal disks. Collected by the writer at Wood's Hole, Mass. Male. × 1.5.

Fig. 9. Transverse section of specimen D, also shown in Fig. 4 (Plate II.). Female. × 2.

Fig. 10. Transverse section of specimen E, collected by B. H. Van Vleck at Cape Ann, Mass. Sex not determined. × 2.

Fig. 11. Transverse section of specimen F, collected by C. W. Prentiss at Wood's Hole, Mass. Male. × 3.

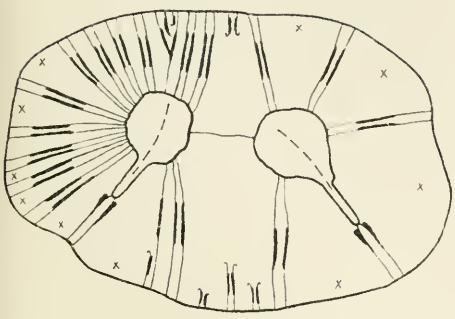
Fig. 12. Transverse section of specimen G, collected by J. I. Hamaker at Newport, R. I. Female. × 2.

Fig. 13. Transverse section of specimen H, collected by R. H. Johnson at Wood's Hole, Mass. Female. × 2.

Fig. 14. Transverse section of specimen I, collected by the writer at Wood's Hole, Mass. Male. × 2.

[Specimen J, collected by the writer at Wood's Hole, Mass., was lost in an attempt to rear it.]

Fig. 15. Transverse section of a natural pair of individuals, showing their mutual relations so far as positions of siphonoglyphs, etc. are concerned. The two specimens, which were entirely separate, were killed and preserved without being removed from the shell on which they were found.



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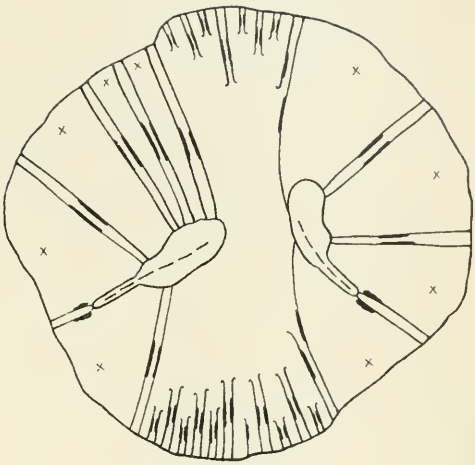

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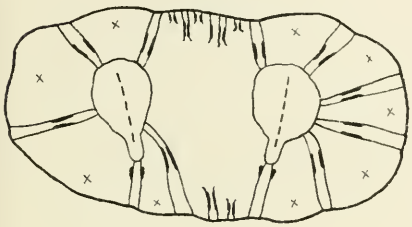

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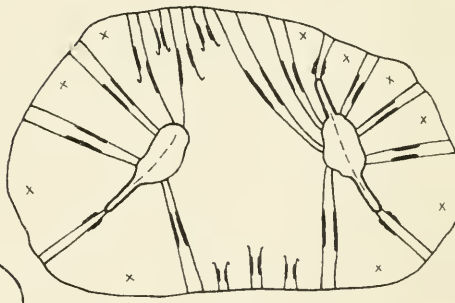

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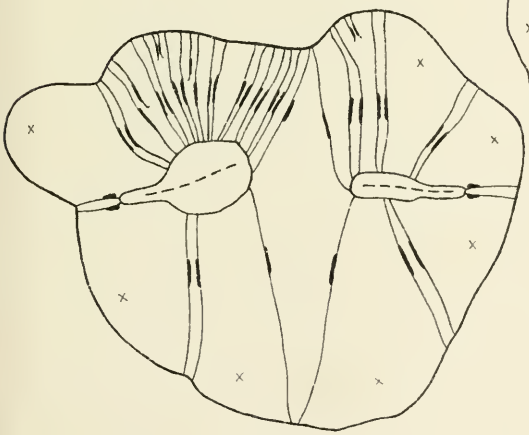

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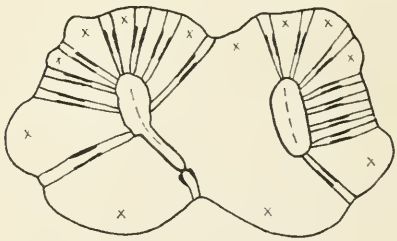

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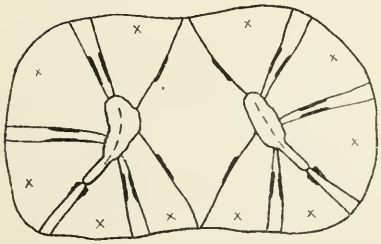

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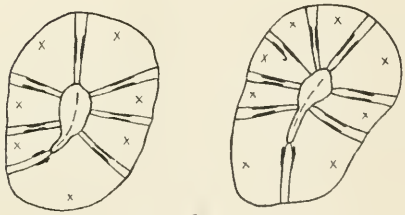

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THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos.

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[3] XV.[16] The Stalk-eyed Crustacea.

S. GARMAN. The Fishes.

W. GIESBRECHT. XVI.[15] The Copepods.

A. GOËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

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C. F. LÜTKEN and TH. MORTENSEN. The Ophiuridæ.

OTTO MAAS. XXI.[21] The Acalephs.

J. P. McMURRICH. The Actinarians.

E. L. MARK. XXIV.[24] The Cerianthidæ.

GEO. P. MERRILL. V.[6] The Rocks of the Galapagos.

G. W. MÜLLER. XIX.[19] The Ostracods.

JOHN MURRAY. The Bottom Specimens.

A. ORTMANN. XIV.[12] The Pelagic Schizopods.

ROBERT RIDGWAY. The Alcoholic Birds.

P. SCHIEMENZ. The Pteropods and Heteropods.

W. SCHIMKÉWITSCH. VIII.[8] The Pycnogonidæ.

S. H. SCUDDER. VII.[7] The Orthoptera of the Galapagos.

W. PERCY SLADEN. The Starfishes.

L. STEJNEGER. The Reptiles.

TH. STUDER. X.[10] The Alcyonarians.

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M. P. A. TRAÜTSTEDT. The Salpidæ and Doliolidæ.

E. P. VAN DUZEE. The Halobatidæ.

H. B. WARD. The Sipunculoids.

H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June, 1891, 16 pp. ; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp

[7] Bull. M. C. Z , Vol. XXV , No 1 September, 1893, 25 pp.

[8] Bull. M. C. Z , Vol. XXV., No. 2. December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4. January, 1894, 4 pp., 1 Plate.

[10] Bull. M C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C Z , Vol. XXV No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV. No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z., Vol XXV. No. 10, October, 1894, 109 pp , 12 Plates.

[14] Mem. M. C. Z., Vol. XVII. No. 3, October, 1894, 183 pp. 19 Plates.

[15] Bull M. C. Z., Vol. XXV. No. 12, April, 1895, 20 pp., 4 Plates.

[16] Mem. M. C. Z., Vol. XVIII., April, 1895, 292 pp., 67 Plates, 1 Chart

[17] Bull. M. C. Z , Vol. XXVII., No. 3, July, 1895, 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol XXVII., No 4, August, 1895, 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895, 14 pp., 3 Plates

[20] Bull. M. C. Z., Vol. XXIX., No I, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M C. Z., Vol. XXIII., No. 1, September, 1897, 92 pp., 15 Plates.

[22] Bull. M. C. Z., Vol. XXXI , No 5, December, 1897, 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M C. Z., Vol. XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[27] Bull. M. C. Z., Vol. XXXV., No. I, July 1899, 4 pp., 1 Plate.

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Reports on the Results of the Expedition of 1891 of the U. S. Fish Commission Steamer "Albatross," Lieut. Commander Z. L. Tanner, U. S. N., Commanding, in charge of Alexander Agassiz.

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OCT 24 1899

Bulletin of the Museum of Comparative Zoology
AT HARVARD COLLEGE.
Vol. XXXV. No. 4.

OVOGENESIS IN DISTAPLIA OCCIDENTALIS RITTER (MS.), WITH REMARKS ON OTHER SPECIES.

By Frank W. Bancroft.

With Six Plates.

CAMBRIDGE, MASS., U. S. A.:
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No. 4. — *Ovogenesis in Distaplia occidentalis* RITTER (*MS.*), *with Remarks on Other Species.*[1] By FRANK W. BANCROFT.

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I. Introduction.

THE Distaplia material here described was collected by Professor William E. Ritter at Pacific Grove, California, during the summer of 1896, and is much of it in an excellent state of preservation. Davidoff's ('89, p. 118) corrosive-acetic mixture, picro-acetic acid, picro-sulphuric acid, and Perenyi's fluid were used for fixing, and of these the first was by far the best, and yielded excellent results.

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. XCVIII.

For the name of the species I am also indebted to Dr. Ritter, who has kindly allowed me to quote his manuscript name, *Distaplia occidentalis*. With the material at my disposal, which, for the most part, consists of only pieces of colonies, and these from but one of the many localities where the species has been encountered, it would be unwise to attempt a complete diagnosis. I will, therefore, endeavor to give only a few of what seem to be the distinctive features.

The colonies vary in shape, being both incrusting and pedunculate; in the latter case they are often mushroom-shaped. The color also varies; in preserved specimens it may be whitish, salmon-yellow, brownish, or purple. The zoöids are small, adult ones exceeding 3 mm. by a few micra only, and they are always hermaphrodite, ovary and testis being well developed at the same time. The ramifications of the pyloric gland do not end in swollen ampullæ. The stomach, though nearly smooth on the outside, is thrown into irregular folds within, so that in whole preparations it has a reticulated appearance. The test is composed of the thin walls of numerous lacunæ. It contains many ectodermic vessels, and these do not anastomose. These characters easily separate *D. occidentalis* from *D. magnilarva* (Delle Valle, '82, p. 200; Lahille, '90, p. 157), *D. lubrica* (Drasche, '83, pp. 22–23), *D. vallii*[1] (Herdman, '86, pp. 128–132), and *D. livida* (Sars, '50, p. 154; Huitfeld-Kaas, '96, p. 11). It appears to be distinguished from *D. clavata* (Sars, '50, p. 154; Huitfeld-Kaas, '96, p. 11) by the great length, 6 cm. of the narrowly clavate colony. This leaves *D. rosea* (Delle Valle, '82, p. 202; Lahille, '90, pp. 174–175; Caullery, '95, p. 8) and *D. intermedia* (Heiden, '93, pp. 348–349), with which our species seems to be closely related. If *D. intermedia* is found to have unisexual zoöids, then this character will separate it from *D. occidentalis*. If, on the other hand, the colonies examined by Heiden were not fully mature, and the zoöids become hermaphroditic later, then his species and *D. rosea* must be united. Thus we get *D. rosea* as the closest, and a very close, relative of *D. occidentalis*. In fact, the only differences seem to refer to characters of minor importance, such as the range in shape and color of the colonies, and the inequalities on the inner surface of the stomach.

[1] If, as Herdman says (pp. 128, 130), *D. vallii* contains no bladder cells in the test which "consists of a homogeneous matrix in which are scattered numerous small test cells and larger pigment cells (Plate XVIII. Fig. 4)," then this species must be considered rather distantly related to the other members of the genus, for in all of these in which the structure of the test has been examined, it has been found to be lacunar.

But, as has already been said, I have not made the extended observations necessary to place beyond doubt the position of *D. occidentalis*.

The Styela and Chelyosoma material was mostly collected by myself, at various places along the California coast, during the summers of 1894 and 1895, and studied about a year later. Four fixing media were used, — Flemming's fluid, picro-sulphuric acid, glacial acetic acid, and Perenyi's fluid, and of these the last gave the best results.

The investigation of the Styela and Chelyosoma material was done for the most part at the University of California, and that of Distaplia entirely at Harvard University during the college years of 1896–97 and 1897–98.

The stains used for the last species were mostly iron hæmatoxylin and a combination of methyl green and acid fuchsin, adapted from Auerbach's ('96, p. 414) method B a. These two methods supplement each other very nicely, and render the use of others almost superfluous; the hæmatoxylin demonstrating most morphological features splendidly, while the chemical constitution is brought out well by the methyl green and fuchsin. The iron hæmatoxylin was employed in the usual way; fresh solutions of the stain were found to give decidedly the best results, but for some things, such as nucleoli, which take a fresh solution too strongly, older ones are often to be preferred. In using the double stain, sections were first treated in a mixture containing two parts of a 0.1% aqueous acid fuchsin solution, to which a little acetic acid had been added, and three parts of a 0.1% solution of methyl green. They were left in this mixture for about fifteen minutes, and then immersed in a 0.1% solution of methyl green for a few minutes longer. From the stain the sections were transferred directly to 90%, and then to absolute alcohol, xylol, and balsam. No especial care is necessary in order to get a satisfactory differentiation. In addition to the two stains mentioned, List's ('96, pp. 480–487) potassium ferro-cyanide methods were also employed, but yielded no very satisfactory results. They will be discussed later.

The sectioning of the younger ova presents no difficulties, but the older ones, which, with the exception of the germinative vesicle, are pure yolk, are exceedingly difficult to deal with, on account of the crumbling of the yolk, and for them no satisfactory method was found. However, all of the material that I had access to had been preserved in 80% or 90% alcohol for some time, so that I did not have an opportunity of testing the effect of preservation in weaker grades of alcohol, formalin or paraffine. As a rule, the whole colony was sectioned, but when dealing

with the maturation stages, the best results were obtained by dissecting out the ova, and then leaving them for a very short time in the higher grades of alcohol, xylol, and paraffine. Four minutes in each of the former, and three in the paraffine, melting at 50° Centigrade, secured a good penetration, but did not entirely prevent crumbling. Painting the sections with flexible collodion was resorted to, but while this held together the section that was being cut, it also pulled part of the yolk out of the next section, and imperfect series resulted. Double imbedding with celloidin and paraffine was also tried, but this made the yolk too brittle, so that I am still in quest of a satisfactory method for this material.

The sections were cut 6 μ thick, and fastened to the slide with tap water, which was evaporated at about 36° after the sections had been stretched by subjection to a slightly higher temperature. This method gave perfectly satisfactory results, and possesses the immense advantage of leaving no foreign substance on the slide.

I wish to express here my thanks to Dr. E. L. Mark, under whose direction this research has been carried on, and to whose constant kindness the credit of much that may be of value therein is due.

II. Sexual Organs.

1. Development in Distaplia occidentalis.

Old colonies which had already reached sexual maturity are the only ones in which I have studied the development of the sexual organs. In such colonies, all of the buds, even the smallest and least differentiated, possess the fundament of the ovotestis. Figure 1 (Plate 1) represents a section through such a bud. The large germ-cells, which indicate the presence of the ovotestis, are situated entirely on one side of the flattened inner vesicle, and all of the evidence indicates that this side is dorsal; for, as soon as the axes of the bud are established by the development of the other organs, the ovotestis is found to lie in the mid-dorsal line. The rather indiscriminate mingling of the young oögonia and primordial follicle cells, and the superficial [1] position of the oögonia as contrasted with the deeper position of the less differ-

[1] Following Julin ('93, p. 95), I use the terms *superficial* and *deep* as relating to the position of the structure in question with reference to the whole individual. *Peripheral* and *central* are used only to describe position with reference to the organ itself.

entiated cells, which will go to make up the testis, are characteristic of all the younger buds. On the other hand, the amount of separation of the ovotestis from the inner vesicle is subject to considerable variation. In the bud figured, the two apparently are fused in places, but in other buds, which are entirely similar in all other respects, the line of demarcation of the ovotestis from the inner vesicle is most distinct. In these cases it is seen that all the mesodermic elements on the dorsal side of the inner vesicle, with the exception of a few scattered cells, are contained in the fundament of the ovotestis. The conclusion, then, to be drawn from the examination of the undifferentiated bud is that the possibility of an endodermic origin for some of the unspecialized cells of the ovotesticular fundament must be conceded; but certainly the greater part, and perhaps the whole of it, is derived from the mesoderm.

So far as the spaces between the cells of the ovotestis are concerned, the figure represents a rather unusual condition, since in most cases the fundament forms a compact mass. But on account of the unusual separation, this bud demonstrates very clearly that in this stage Distaplia has no "assise périphérique" such as Julin ('93, p. 97) and Floderus ('96, p. 175) found in the youngest stages examined.

In slightly older buds, where the fundaments of the intestine and epicardium are present, the only change in the ovotestis is due to a few of the ova having increased in size and assumed more distinctly the characters of the oögonium. Its position is still dorsal, but it occupies only the posterior end of the dorsal side of the bud. At still later stages, even after the peribranchial sacs are quite large, the ovotestis is apparently fused with the posterior end of the dorsal tube. This connection is often very puzzling; in one series, for instance, the lumen of the dorsal tube extends quite to the ovotestis, the latter appearing as a thickening of the dorsal tube. In buds of these stages there is considerable variation in the disposition of the organs, the ovotestis being placed slightly to one side of the dorsal tube or directly behind it, or even dorsal to its posterior end. Accordingly, I am inclined to believe that the apparent union between these two organs is not significant, but due rather to their close approximation and to oblique sectioning. This position is further strengthened by the fact that both before, immediately after, and even occasionally during, this stage, the ovotestis is completely isolated from all other organs. Whenever the ovotestis is thus isolated, it is produced anteriorly into a genital strand, which occupies very nearly the mid-dorsal line. It seems likely, then, in view of the very general isolation of the sexual

organs observed in the early stages, that the whole of these organs is produced by the growth of the ovotesticular fundament of the undifferentiated bud.

In older buds the genital organs are always distinctly separated from the other organs, and the differentiation of ovary from testis is speedily accomplished. Figures 2 and 3 (Plate 1) represent two consecutive cross-sections through a stage in which this separation is going on. In Figure 2, the posterior section, there are two well-defined masses of cells which in Figure 3 are united, the only indication of the future separation being a slight notch on one side of the fundament. The splitting takes place from behind forwards, and appears to be accomplished by a rearrangement of the cells in situ, and the secretion of a structureless membrana propria between the two masses. Preceding the actual separation, however, there is a differentiation consisting in the fact that the smaller cells, which will produce the testis, are always situated deeper than the others, and this, as Figure 1 shows, is characteristic even of the earliest stages. Both ovary and testis are usually solid masses of cells at this stage, and for some time later, but occasionally one or both of them may develop a lumen (Plate 1, Fig. 3), though in the case of the testis it is never well defined.

Figure 4 (Plate 1) gives a longitudinal section of a somewhat later stage, the ovary and testis being completely separated from each other, but both still attached to a common genital strand (*fun. gen.*). The latter extends from the anterior ends of the genital organs to the left peribranchial sac, with the walls of which it fuses. It is much thicker and shorter in the earlier than in the later stages, when the distance that it has to traverse becomes greater.

An examination of Figures 2, 3, and 4 shows that at this stage there is a well-defined tendency for the long axis of the peripheral nuclei of the testis or testicular part of the ovotestis to occupy a tangential position. This marks the beginning of the peripheral epithelium that bounds the testis at all later stages. In the ovary, however, such a peripheral layer is absent in this stage, as in the earlier ones, so that the young oögonia or their follicles extend to the surface of the organ. This is exactly the condition found by van Beneden et Julin ('85, p. 334) for the same stage of these organs in Perophora. Their figures (6 *a* and 6 *b*, Planche XVI.) show the peripheral layer well differentiated in the testis, but not in the ovary, though the two organs are still connected.

The fundaments of the oviduct and vas deferens are at first formed

by the splitting of the genital strand, which is progressively converted from behind forward into vas deferens and oviduct. But with the growth of the bud, the undifferentiated portion of the strand is stretched more and more, until it becomes a single row of cells attached end to end, which connects the last differentiated portions of the two ducts with the peribranchial epithelium.

From this time on cell-multiplication must take place pari passu with the separation of the ducts. But as no mitoses were encountered, it is useless to speculate as to whether this multiplication is accomplished in the region of the genital strand, the differentiated ducts, or the mass of cells where the three join. Even before the ducts are separated throughout their whole length a histological difference is apparent, the nuclei of the oviduct cells being more flattened and taking a fainter stain than those of the vas deferens. Another point of interest is that, contrary to what obtains in other species, both ducts are solid strands, which do not acquire a lumen until much later.

While the ducts are being separated, the testis increases in size and entirely covers the deep side of the ovary, so that in cross-section it appears as a crescent, the tips of which abut against the ectoderm on either side of the ovary. Later it becomes divided up into the eight or more testicular pouches which are characteristic for the species. Meantime the ovary increases in size and acquires a lumen, on the deep side of which most of the ova are situated. With these last changes all the essentials of the adult structure are reached, but the position of the sexual organs with respect to the axes of the animal, and the point of union of the genital strand with the peribranchial sacs, still undergo some change.

In young buds with small peribranchial sacs, which have not yet united to form the median cloaca, and without a trace of stigmata, the sexual organs are situated in the mid-dorsal line, and also in the intestinal loop which has developed around them. At this stage the œsophagus opens into the right posterior corner of the branchial sac and the anus is near the left posterior corner, so that the whole of the post-pharyngeal digestive tract is in a frontal plane. The genital strand extends forward in the median line and joins the tip of the left peribranchial sac, where this comes nearest to the sagittal plane. Later the point of union of the genital strand with the peribranchial sac migrates still farther to the left, so that it comes to lie much nearer to the anus. Now, as the bud grows and assumes the adult shape, the whole abdomen is so twisted that right becomes ventral, left dorsal,

and the loop of the digestive tract occupies the sagittal plane. By means of this twisting, the anus becomes dorsal and the point of union of the genital strand with the peribranchial sacs migrates from the left through the cloaca to the right peribranchial sac; and thus the adult relations of the organs are acquired.

Conclusions.

From this account it follows that the development of the sexual organs in Distaplia occidentalis agrees with that of the three species described by van Beneden et Julin ('85, pp. 328–349) and of Ciona intestinalis described by Floderus ('96, pp. 173–181) in the following important respects: —

1. The whole of the sexual organs of the adult is developed from a single solid mass of cells of mesodermic origin, which during the later period of its existence is connected with the cloaca by a genital strand.

2. The ovary is separated from the testis by a splitting of the primitively single fundament which proceeds from behind forward. The separation of the two ducts takes place in the same manner, though here growth must intervene. The ovary and oviduct always lie on the superficial side of the testis and vas deferens.

3. The cavity of the ovarial fundament is formed in such a way that the germinative epithelium lies in its deeper wall.

Distaplia occidentalis differs from the species mentioned in that: —

1. The ovary and testis are usually solid, and the ducts always so, at the time when they are separated from each other.

2. The genital strand is at first quite thick, and decreases in diameter as the bud grows.

3. The fundament of the ovotestis is present in the youngest stages, whereas in the other species it appears quite late in ontogeny.

With the development of these organs in Styelopsis grossularia studied by Julin ('93), that of our species has less in common, because in the two the ovaries and testes are of a different type. In Distaplia, and the four species with which it has been compared, the ovary is distinctly separated from the oviduct proper, in the walls of which no ova arise, while in Styelopsis the deep wall is given over to the production of ova throughout the whole extent of the oviduct. Furthermore, in Styelopsis the testis is divided into a number of lobes, each one opening separately into the peribranchial cavity, whereas in the other species it is a single organ with a single long duct. Accord-

ingly, there is no genital strand at all in Styelopsis, and the separation of ovary from testis takes place from the middle towards both ends; in the other respects mentioned, however, this genus agrees with Perophora, Phallusia, Clavelina, and Ciona.

With respect to the presence of a peripheral epithelium surrounding the developing ovotestis, there is more variation. In Perophora and Distaplia this is present in the testis but not in the ovary, while in Clavelina, according to the figures of van Beneden et Julin ('85, Planche XV., Figs. 9, 10, and 13), in Styelopsis and in Ciona it is encountered in both. This distribution is very suggestive, for the first two genera have rather small zoöids, and a simple structure, while the last three are much larger, produce many more sex cells, and are more highly specialized. It seems, then, that it is only in the larger and more complex ascidians that this peripheral epithelium is formed around the developing ovary.

For the explanation of the fact that the oögonia are present in the undifferentiated Distaplia bud, we must undoubtedly look to the fact that in this genus, as in Botryllus, the life of the individual zoöid is very short, so that apparently it would not have time to mature an ovum if the latter were to start from a wholly undifferentiated cell, and consequently it receives the ovum in a slightly elaborated form. In these two genera, however, the structure and development of the sexual organs seem to be entirely different, as according to Pizon ('92–'93, pp. 257–271), the ovary and testis of Botryllus arise from separate fundaments, and no real ovary is formed at all, but merely a few follicles attached by separate stalks to the peribranchial sacs.

Distaplia also agrees with Botryllus and disagrees with all other species in which the matter has been studied in that the fundaments of the sexual organs and their ducts are usually solid. This condition is probably the direct result of the early development of these organs necessitating their being blocked out in solid masses before enough cells to form vesicles were available.

On the whole, however, it is evident that the development of the sexual organs in our genus conforms to the type first described by van Beneden et Julin for Perophora, Clavelina, and Phallusia, and deviates rom it only in minor respects due to the simpler structure and short flife of the zoöids in Distaplia.

2. Structure in the Adult.

a. In Distaplia occidentalis.

This species of Distaplia is always hermaphroditic, no unisexual zoöids such as have been described for D. magnilarva having been encountered. Nor is there any provision for the prevention of self-fertilization by means of protandry or protogyny. The testis is found to be functionally active for a much longer period than the ovary, for spermatozoa are always found in the vas deferens from the time the oldest ova are about half grown until after the usual number of eggs, two, has been laid.

The development of the genital organs in zoöids of the same size varies considerably according to the condition of the colony. If the oldest zoöids of a colony are laying eggs or have recently done so, then all the younger ones and the older buds have their sexual organs more fully developed than in individuals of the same size in colonies which possess no mature sexual products. In the undifferentiated buds, however, the same conditions obtained in all the colonies.

The position of these organs is on the right side of the intestinal loop. When partially developed, they do not extend beyond the intestine, either anteriorly or posteriorly, but when maturity is reached, they project beyond it in both directions. In this condition the ovary occupies the extreme hind end of the zoöid and is entirely posterior to the loop. The testis, which is much more voluminous, is also mainly posterior to the loop, but its extreme anterior end is usually within it.

Concerning the finer structure of the ovary, recent investigators are not entirely in accord. Corresponding to the fact that in the development of this organ a peripheral epithelium is described by Julin and Floderus, which the earlier investigators had not mentioned, there is a similar disagreement about the adult structure. But here the difference is of much more importance, for Julin, in tracing the peripheral membrane into the adult ovary, arrives at an idea of the relation of the germinative epithelium to the peripheral wall essentially different from that of his predecessors. Van Beneden et Julin ('85, pp. 350, 358) and Maurice ('88, p. 456, 464) had found that the superficial wall of the ovary passed insensibly into the germinative epithelium occupying the lateral edges of the organ. The deeper[1] wall, which is

[1] There can be no doubt that in van Beneden et Julin's Figure 14 (Planche XV.) the wall of the ovary of Clavelina rissoana that is placed uppermost and described

formed by a cylindrical epithelium in both Clavelina rissoana and Fragaroides, and to which the stalks of the older follicles are attached, is considered as having developed from the germinative epithelium. According to this view, then, both the germinative epithelium and the cylindrical epithelium connecting with and forming the stalks of the follicles[1] are merely differentiations from the wall of the ovary and not additional structures. In other words, the ovary is but a specialized part of the oviduct.

On the other hand, Julin (p. 95) finds that in Styelopsis the superficial wall of the ovary connects with a thin limiting epithelium and not with the germinative epithelium. The latter represents an additional element lying within the cavity formed by the two peripheral epithelia and not represented at all in the *oviduct* of such forms as Clavelina and Fragaroides. As the ova increase in size, they push the limiting epithelium before them, and retain their connection with the rest of the ovary by means of it. Thus, according to this author, the outermost layer of cells surrounding the ovum and continuous with the stalk is not, as has been formerly supposed, derived from the germinative epithelium, and is not part of the true follicular covering of the egg.

Floderus, though he finds a limiting epithelium in the young ascidian, does not agree with Julin concerning the structure of the adult. He finds that the germinative epithelium continues into the epithelium of the superficial wall (p. 187), that the epithelium which he thinks corresponds to Julin's limiting epithelium does not connect with the superficial wall (p. 185), and that the stalks of the ova connect with their follicles (p. 190).

But little evidence in favor of Julin's contention has been found in the ovary of Distaplia occidentalis, which has essentially the structure

(p. 351) as "la voûte de cette même cavité" is the deep or ventral wall. Maurice (p. 459) takes it for granted that this wall is dorsal, and points out the difference that would exist between Clavelina and Fragaroides on this point. Floderus (p. 183–184) discusses the question fully and concludes that although van Beneden et Julin do not state explicitly which side is dorsal, the description and the position of the figure when compared with the other figures on the plate compel us to believe that the authors consider the "voûte" dorsal. However, leaving the question of the intention of the authors aside, the ventral position of the germinative epithelium in the *fundament* of the ovary in C. rissoana and of the ovarian follicles in the adult ovary of C. lepadiformis, described by Floderus, point conclusively to the fact that the same condition must obtain in the adult C. rissoana.

[1] I propose in future to call the characteristic tissue constituting this epithelium stalk tissue.

described for the same organ in Fragaroides. It consists of two sacs, the first of which is an enlargement of the posterior end of the oviduct, and whose cavity may be designated as the lumen of the ovary proper (Plate 1 Fig. 5, *lu. ov.*). On the deeper wall of this sac is situated the germinative epithelium, which occupies the greater part of its extent. When viewed from within the animal, this epithelium presents an oval outline with its long axis running in an antero-posterior direction. The second sac, whose cavity is spoken of as the lumen of the stalk tissue, is situated on the deep side of the first one, and its lumen connects with that of the ovary proper by a narrow passage in the stalk, which is inserted in the deep wall of the ovary proper at about the centre of the oval patch of germinative epithelium. Figure 5 represents a cross-section through both these cavities and the stalk connecting them.

In our species the adult ovary is composed of three tissues (Plate 1, Figs. 5, 6). First the thin pavement epithelium comprising the superficial[1] wall of the organ (*par.'*). Usually it forms also the lateral edges and is often continued so as to constitute part of the deep[1] wall (Plate 1, Figs. 5, 6, 7, *par.''*). It is directly continuous with, and of the same histological structure as the wall of the oviduct, both being very thin and devoid of cilia.

Secondly, the germinative epithelium and the older ovarian follicles developed from it. The former sometimes appears as directly continuous with the superficial wall, but more often, especially when the ova are a little larger (Figs. 5, 6, 7), they seem to lie enclosed within a peripheral epithelium which is itself continuous with the superficial wall. The ova, which are the only indication of the germinative epithelium, are very few, the total number in an adult zoöid being usually under twenty. Occasionally a very small oögonium, or an ovum that may perhaps be considered undifferentiated (Fig. 5, *ov'go.*), is found within the superficial wall, but in the great majority of cases they are confined to the deeper wall. Here they are usually so arranged that the older the oögonium the nearer it is to the middle of the wall to which the stalk tissue is attached (Fig. 5). There are very few or no primordial ova in the epithelium, so that physiologically it is really no germinative epithelium at all, but merely a repository for the youngest oögonia. It is very interesting to note, however, that this repository of oögonia takes on the same relations to the rest of the ovary that the functional germinative epithelia of the more specialized

[1] Used in the technical sense defined on page 62.

genera assume. In Distaplia occidentalis this arrangement of the oögonia, and even their existence there, has reference to the phylogeny of the genus rather than to present usefulness, for the usual number of eggs laid by a zoöid is two, or at most three, and since in the adult there is no provision for budding, all the younger oögonia must degenerate with the zoöid and go to waste.

Thirdly, there is the stalk tissue (Figs. 5, 6, *tis. pd.*), which is directly continuous with the germinative epithelium or its peripheral membrane, and also by means of the follicular stalks with the follicles of all the older ova (Plate **3**, Figs. 14, 15, *tis. pd.*). This, in its typical condition (Figs. 5, 15), is a cylindrical epithelium with rather irregular oval nuclei and a thick, structureless basement membrane on its peripheral surface. The stalk tissue surrounds a cavity (Fig. 5, *lu. pd.*) that is usually somewhat larger than the true cavity of the ovary, with which it connects by a narrow stalk. This stalk joins the rest of the ovary in the region of the germinative epithelium, so that a series of cross-sections presents exactly the same appearance as in Fragaroides. Posteriorly the deep wall is composed of a continuous germinative epithelium; about the middle of the epithelium the cavity of the stalk tissue connects with the cavity of the ovary proper and divides the germinative epithelium so that in a cross-section of this region it looks like a paired structure (Plate **1**, Fig. 5). Anteriorly the entire centre of the deep wall is made up of germinative epithelium as it is posteriorly. As a result of this structure, it is seen that the stalk tissue does not connect with the flat pavement epithelium comprising the wall of the oviduct. The cavity of the stalk tissue extends posteriorly behind that of the ovary proper, and has the follicles attached to it in such a way that the oldest ovum is usually the most posterior one. From this account it follows that the ovary, though not a paired organ, is a symmetrical one; but even this is not always the case. Occasionally the stalk tissue, instead of being inserted near the centre of the deep wall, joins the latter near one side, so that it connects with the superficial wall on one side and the germinative epithelium on the other.

The question as to whether in Distaplia occidentalis there is a limiting membrane which is distinct from the germinative epithelium, seems to be answered negatively. The condition mentioned above, and represented in Figures 5, 6, and 7, where the ova project strongly into the cavity of the ovary, seems to indicate that the germinative epithelium is bounded peripherally by a limiting membrane.

But it is impossible at this stage to tell whether the side of the ovum next to the periphery of ovary is covered by but one cell layer, the follicle, or by a limiting epithelium in addition to this. I have never been able to differentiate a limiting epithelium in this position, but the failure could not justify one in denying its existence. When it comes to the formation of the follicular stalk, however, evidence is obtained that is conclusive, for we get the external epithelium of the ovary, which should extend around the ovum independently of the follicle, connecting with it in the clearest possible manner (Plate **3**, Figs. 14, 15). Figure 14 shows part of a young ovum that is entirely outside of the ovarian wall, but which is not yet attached to it by a stalk. On the left the ovum is attached to the germinative epithelium, as the tangential section of a much younger egg (*ov'go.*) shows. On the right it joins stalk tissue which has not yet assumed its typical appearance. Between the two are cells which make up both the follicle and the wall of the ovary, and will probably differentiate into follicle and stalk tissue. Figure 15 represents the same region in an older ovum, where the stalk tissue presents its characteristic structure and is seen to be directly continuous with the follicular epithelium. From these facts the conclusion seems justified that in the adult Distaplia ovary, just as in the developing one, we have no peripheral limiting epithelium.

One feature that is not usually encountered in other species, but is quite conspicuous in the ovary of this one, is the corpus luteum (Fig. 5, *cp. lut.*). This structure is merely the part of the follicular covering of the egg which remains behind when the latter is extruded from the ovary. It will be described in detail after the origin of its constituent elements has been considered.

The oviduct (Plate **2**, Fig. 9, *ov'dt.*) is a narrow, thin-walled tube extending up the right side of the abdomen and in immediate contact with the ectoderm. Throughout its extent the vas deferens (*va. df.*) is closely appressed to its deep wall. The most striking peculiarity of the oviduct is that its diameter, even when distended by the passage of an ovum, is very much less than the normal diameter of the ripe egg. Accordingly, when the egg is passing through the duct, it is greatly distorted, assuming the shape of a sausage (Plate **3**, Fig. 17). In this figure the ovum occupies a part of the oviduct, all of the cavity proper of the ovary, and also the entire cavity enclosed by the stalk tissue and its own follicle. The point where the stalk tissue joins the germinative epithelium lining the deep wall of the ovary

proper is well indicated by the constriction at *pd*. Figure 16 shows the normal size of the ovum before laying.

b. Observations on Other Species.

I have examined sections of the adult ovary in several other species, and describe the condition in two of these, on account of the light it throws on our conception of the fundamental structure of the ascidian ovary.

The first of these species is Styela montereyensis,[1] whose ovary presents a structure similar to, but not identical with, that described for Styela rustica by Floderus (p. 185–186). The ovaries are four elongated tubes, two on each side, which open at their anterior ends into the atrial cavity. The superficial wall is composed of a ciliated epithelium, which connects with a germinative epithelium at the lateral edges. The deep wall is much folded in an irregular manner, and is composed of a thin pavement epithelium with occasional small oögonia contained in its thickness. At times portions of the deep wall, where these oögonia are crowded together, look a good deal like germinative epithelium. I am convinced, however, that this is not the case, for nowhere in the deep wall are oögonia of the smallest size and undifferentiated ova, such as exist in the two lateral germinative epithelia, encountered. The presence of these small oögonia outside the germinative epithelium is due to the fact that the latter sometimes gives off its oldest oögonia after the younger ones, which are thus carried out into the deeper wall before they are really ready, and remain in it for a considerable period, while they are reaching the size at which they project beyond the pavement epithelium. All the older oögonia are situated on the deep side of this epithelium, presumably attached to it by their follicular stalks, though these are but seldom seen. In each of these four ovaries, then, we have essentially the same structure that exists in Clavelina and Styelopsis, where the ovary is single and situated in or near the sagittal plane. Furthermore, it is likely, though not proven, that these four ovaries are developed from separate fundaments, for in a very much younger stage (Plate **2**, Fig. 12), when the whole of the deep wall is made up of the germinative epithelium, and the primordial ova are still dividing,

[1] This is one of the commonest of our California ascidians. It was first described by Dall ('71, pp. 157, 158) as *Cynthia montereyensis*, and subsequently by Fewkes ('89, pp. 134, 135) as *Clavelinopsis rubra*. Ritter ('93, p. 39) placed it in its proper genus, *Styela*.

and the vas deferens opens into the oviduct instead of the atrium, these organs occupy the same relative position as in the adult.

The second species is Chelyosoma productum, Stimpson ('64, p. 161). Here the structure is similar, but not identical, to that described by Floderus (p. 187) for the genera Ascidia, Ascidiella, and Corella. The ovary is much branched, occupying the superficies of the viscera, and also extending in between the stomach and intestine and covering their deeper surface. In each lobe, however, the conditions are different from those in the genera mentioned, in that the germinative epithelia are in two strands, which are usually entirely separated from each other except at the ends of the lobes, where they fuse. In the great majority of cases, especially where the lobes are superficial, they are so flattened that they are parallel to the surface. One of the walls thus formed, which is usually the superficial one, has its median part formed of a cubical epithelium with long cilia, and on both sides of this are situated the germinative epithelia with the largest ova away from the median line. Occasionally the germinative epithelia are broad enough to reach the lateral edges, but these are usually formed by the ova that are just large enough to extend beyond the wall of the ovary. The other wall is made up of the same kind of ciliated epithelium, and to it all the follicles of the older ova are attached. Here again, then, we have the essential structure of Clavelina and Styelopsis, but developed in each of the lobes of an irregularly branching ovary. It must be said, however, that although the structure of the two walls is quite constant, their position with respect to the surface of the animal is somewhat irregular. The position is most constant in the superficial lobes, but even here only about 76% have the wall containing the germinative epithelia superficial, while in about 14% it occupies the deeper position; and in the other 10% the plane of the lobe is approximately at right angles to the surface. In the lobes that extend between the stomach and intestine, and over their deeper side, the variation in orientation is still greater, depending more upon the adjacent organs than upon the surface of the animal.

c. General Considerations.

Floderus (p. 250) established the law that in forms with bilateral arrangement of the germinative epithelia the development within the same takes place from without inward. In general this holds good for the three species studied, though some exceptions are encountered. It would be equally true to extend the law from the germinative

epithelia to the whole ovary, but the case of the deeper lobes of the Chelyosoma ovary shows that its applicability is limited to ovaries, or their parts, that have a superficial position. Stated in its most general form, then, the law of growth within the ascidian ovary of the regular type seems to be that:

In general, in ovaries or parts of ovaries having a superficial position and bilateral symmetry, whether the germinative epithelium is double or not, development takes place from the median line of the superficial wall towards the deeper parts of the animal.

Another general question is whether the Clavelina type of ovary, with two separate germinative epithelia, should be considered more primitive than that of Distaplia and Fragaroides, where a single germinative epithelium occupies the greater part of the deep wall. It seems to me that the condition in Distaplia is more primitive, for it occurs in smaller and simpler species, and is in itself a simpler condition. Furthermore, this contention is borne out by the young Styela ovary (Plate 2, Fig. 12), where the germinative epithelium occupies the whole deeper wall of the ovary, though it is double in the adult. It is, however, possible that both the condition in Fragaroides and Styela may be the result of a secondary fusion; and Julin's work on Styelopsis, where the germinative epithelium is formed from two single rows of cells extending along the lateral edges of the ovarian fundament, and proliferating primordial ova and oögonia towards the middle line, favor this view. In Styelopsis the two epithelia thus formed do not come near enough to fuse, but it may be that they do in other species. In Distaplia, however, there is certainly no fusion, for the germinative epithelium is single from the first. There is about the same number of oögonia in the undifferentiated bud that there is in the adult, so that the whole ontogeny of these cells consists in rearrangement and growth. A few of these oögonia commence to increase in size at a very early period, and, as these are usually near the middle of the deeper wall, it is here that the first follicle and consequently the stalk tissue is formed. But we should expect that occasionally an ovum near the edge would commence to grow first, and accordingly we actually do find that in a few cases the stalk tissue is attached near the lateral edge of the ovary. In fact, from the study of the development in Distaplia, one gets the impression that the bilateral symmetry is accidental, depending upon the fact that usually the first ovum to grow will be somewhere near the centre of the deep wall. But that this cannot be true for other species, and may not be true in this one, is

shown by the fact that in Clavelina, Ciona, and Styelopsis the doubling of the germinative epithelium occurs before the oögonia are formed.

III. Incubatory Pouch.

1. Historical Summary.

Almost all compound and social Ascidians have some provision for retaining their eggs within the colony until the larvæ developed from them are ready to enter upon their free swimming existence. The organ where this is accomplished is usually called an incubatory pouch, but its structure varies much in different groups. Usually the pouch is merely a slight enlargement of one peribranchial sac, into the bottom of which the eggs are laid, and from whose top the fully formed larvæ escape. Occasionally, however, as in Glossophorum sabulosum described by Lahille ('90, p. 203), the pouch consists in the terminal enlargement of the oviduct. Distaplia and Colella, and probably also the allied genus Julinia, Calman ('94), present an extreme development of incubatory pouch, which consists of a large diverticulum connecting with the dorsal edge of the zoöid by a narrow stalk. Within the enlarged terminal part of the pouch the eggs and embryos are always arranged so that the youngest are at the bottom; but how they got there has been somewhat of a puzzle to ascidiologists. Delle Valle ('81) published the first account of this structure; he supposed that the ripe ova reached the peribranchial sac and were put into the pouch together, and then fertilized. The retardation that the spermatozoa would experience in travelling down the pouch and getting past the first ova he thought accounted for the greater development of the embryos near the mouth of the pouch. The next investigator of the subject was Herdman ('86), who studied this organ in Colella pedunculata and allied species. He describes the pouch (p. 89) as "merely an enormous diverticulum of the peribranchial or atrial cavity" and remarks that the neck is so narrow that ova can pass in but that the larvæ cannot escape. He explains the arrangement of the embryos within the pouch by supposing that the ripe eggs all reach the peribranchial sac, are there fertilized and subsequently put into the pouch in order, the one last fertilized going first. But he says that he has no evidence for this, as eggs have never been found in the peribranchial sac. Since then no investigator, so far as I know, has attempted to account for the arrangement of embryos in incubatory pouches of the

Distaplia type. Calman ('94, p. 10), however, in describing the genus Julinia, which is closely related to Distaplia, gives a description of a spherical vesicle attached to the dorsal side of the thorax which he believes to be an incubatory pouch, though no eggs or embryos were found in it. The most significant thing about his account is that the lumen of the oviduct seems to be continuous with that of the pouch.

2. Observations.

The incubatory pouch in Distaplia occidentalis is not so large as in some other members of the genus. It usually contains two embryos, and never more than three, according to my experience, while in D. magnilarva, according to Delle Valle's figure ('82, Fig. 5), the pouch may contain as many as eight. The conditions vary in different colonies; in some the pouches contain but one or two embryos, while in others three is the predominating number. The pouch is attached to the posterior dorsal region of the thorax, a little to the right of the median line, and when fully formed, though still attached to the zoöid, it extends posteriorly about as far into the colony as the zoöid itself.

A careful examination of the structure of the pouch shows that it is not merely a diverticulum from the peribranchial sac, but consists of two parts which, for descriptive purposes, may be called the oviducal and the peribranchial portions, though I do not know that they have been developed from the oviduct and peribranchial sac respectively. The oviducal part is a narrow tube, the anterior end of which connects with the oviduct, and the posterior end with the bottom of the pouch. Anteriorly the peribranchial portion is a narrow tube opening into the posterior dorsal corner of the right peribranchial sac. Posteriorly, however, it is enlarged to form the pouch proper, in which the developing embryos are lodged. Figure 8 (Plate 2) represents a young pouch in which the relation of the oviducal (*brs. ov'dt.*) to the peribranchial portion (*brs. pi'brn.*) is shown. Both portions are of course covered by the evaginated ectoderm (*ec'drm.*). The relations of the stalk of the pouch to the zoöid are best shown by means of cross-sections (Plate 2, Figs. 9, 10, 11). The most posterior section (Fig. 9) shows the stalk entirely separated from the zoöid and imbedded in the common test. Its oviducal portion (*brs. ov'dt.*) is seen to be nearest to the part of the zoöid containing the oviduct (*ov'dt.*). As the series is followed anteriorly, the ectoderm of the stalk first joins that of the zoöid and then the wall of the oviducal portion becomes continuous

with that of the oviduct (Fig. 10, *ov'dt.*). Figure 11 is taken from another zoöid and shows the opening of the peribranchial portion of the stalk into the right peribranchial sac. As the pouch is completely separated from its zoöid long before the larvæ are mature, the only function of this peribranchial orifice is to serve as a passage for the spermatozoa. The vas deferens (*va.df.*), which is closely applied to the deep wall of the oviduct until the latter joins the oviducal part of the pouch, extends much farther forward and opens into the cloaca near the anus. It is thus seen that the oviducal portion of the pouch is a continuation of the oviduct into the pouch, and that the egg never reaches the peribranchial sac at all, but is conveyed directly to the bottom of the pouch.

The lumen of the oviducal part of the pouch is even narrower than that of the oviduct, so that here too the egg is greatly elongated in its passage. Figure 18 (Plate 3) shows an egg the greater part of which has just entered the pouch; but a small portion of it has not quite emerged from the oviducal tube, and can be seen as a small projection from the rest of the ovum. From the fact that there are no muscles present in the pouch, and none but the heart muscles in the abdomen, and because the ovum has shrunken away from the walls of its conducting tube in preserved specimens, I believe that this compression is not due to the killing reagent, but represents a normal condition.

I have not been able to discover the pouch at the very beginning of its development, and so cannot say whether it is formed as an evagination from the peribranchial sac, from the oviduct, or from both. It is probable, however, that even if the first stages were found it would be difficult to settle this matter, so that a comparative anatomical investigation of the subject in Colella, for instance, where all grades of pouch formation obtain, would be more profitable. I have, however, found the fundament when it was only a short cylindrical outgrowth about 150 μ long. At this stage it presents all the essential features of the adult except that the pouch proper is very little enlarged. In fact Figures 9 to 11 (Plate 2) were taken from a stage but little older than this. Zoöids with pouches in this condition are about 2 mm. long, that is about two-thirds grown. The vas deferens, however, is filled with spermatozoa apparently ready to be discharged. Subsequent development consists in a swelling of the pouch, and an elongation of its stalk, so that the whole structure moves posteriorly into the colony at some little distance from the zoöid producing it. When the bottom of

the pouch is at about the level of the stomach, the first ovum enters it; when the last ovum has entered, its end is about at the level of the posterior extremity of the zoöid.

None of the pouches containing the older embryos are ever found connected to zoöids, and as no adult zoöids are ever encountered without pouches, it is evident that the zoöids from which the older pouches arose have degenerated, and that those found in the colony along with these older pouches belong to a subsequent generation. As a matter of fact, degenerating zoöids are not infrequently encountered, and in close proximity to them are seen the pouches with which they were probably connected. Most of the growth of the embryos takes place after the degeneration of their mother; accordingly the pouch is much enlarged, becoming even longer than the adult zoöid was, and the narrow anterior stalk apparently swells up to the size of the pouch proper. The whole structure migrates through the test until its anterior end finally comes into close connection with one of the common cloacal cavities, through which the fully developed larvæ reach the exterior.

IV. Envelopes of the Ovum.

So many summaries of this subject have been published, and such a good one by its most recent investigator, Floderus, that a detailed discussion of the literature would be superfluous. In fact the whole subject has been left in a very satisfactory condition by this author, who was the first to discuss the evidence for the formation of the follicle cells and test cells, both from within the ovum and from sources which are external to it. Accordingly, much that I have determined is confirmatory of his results, and of those of earlier investigators. However, as it was from the genus Distaplia that Davidoff ('89) drew what is probably the strongest evidence for the intraovular origin of the test cells, it seems worth while to discuss the conditions in this genus.

1. Primitive Follicular Epithelium.

Concerning the development of the primitive follicular epithelium (van Beneden et Julin, '85, p. 357), I can confirm the account of Floderus and all the other investigators of the subject since van Beneden et Julin's time. The epithelium is formed from the primordial follicle cells found in close proximity to the oögonia in the earliest stages

(Plate 1, Figs. 3, 4; Plate 2, Fig. 12, *cl. fol. pr.*). Even in the undifferentiated bud (Fig. 1), there are found among the oögonia smaller nuclei that will probably give rise to nuclei of follicle cells. As the oögonium increases in size, these cells multiply and form a continuous epithelium around it (Plate 1, Fig. 7; Plate 3, Figs. 21, 23, 13). Julin ('93, pp. 106–109) states that the first follicle is composed of three cells, one of which is the sister, and the others the products of division of the cousin of the ovum; and also (p. 123) that the entire follicle of later stages is derived from these three cells. In our species, where the ovogenesis is not so rapid, and there is plenty of time for a rearrangement of the cells, there is no evidence showing that the formation of the follicle is so precise; and the fact that in one instance a small ovum has been found in the follicle of an older one (Plate 3, Fig. 26) seems to show that the follicle is made up from any cells that happen to be in the vicinity of the growing oögonium.

The stalks connecting the younger follicles to the germinative epithelium and the older ones to the stalk tissue are, as has already been shown, differentiations from the germinative epithelium which connect with the follicle, and are not derived from a limiting epithelium of the ovary. However, even before the stalk of the follicle is completely established the differentiation of the primitive follicle into secondary follicle cells and test cells has begun.

2. Test Cells.

Davidoff, in studying the origin of the test cells in D. magnilarva, found evaginations of the wall of the germinative vesicle, and concluded that these are constricted off to form the vesicular structures present in the cytoplasm of the ovum. These vesicles, he says, sometimes appear empty and sometimes have a chromatic spot within them. Their peripheral membrane stains very lightly, but as they move towards the periphery of the ovum, they progressively take a deeper stain and develop chromatic granules until they become the fully developed nuclei of the test cells. Later their cytoplasm is formed from portions of the cytoplasm of the ovum. Caullery ('94, p. 600), who worked on the ovogenesis of D. rosea, does not confirm Davidoff's results, but says that the test cells arise from the follicular cells by mitosis. He does not, however, give the evidence upon which this statement is based.

a. Evidence for the Intraovular Origin of the Test Cells.

I have observed much of the evidence upon which Davidoff bases his account, but do not consider it strong enough to establish his contention. As a rule the wall of the young germinative vesicle is perfectly smooth (Plate 3, Figs. 20–23, 13), but in quite a number of cases it is thrown into folds. A rather extreme instance of this folding is shown in Figure 19 (Plate 3), which, however, represents well the most characteristic feature, which is that the folds usually occur at the ends of an oval germinative vesicle. The long axis of the germinative vesicle is often parallel to the edge of the knife used in sectioning, so that in these cases the folding may be due to the shoving of the section in cutting, but in other cases this cause cannot be invoked. Davidoff's figures represent the folding as being most pronounced at the ends of the germinative vesicle, but not limited to that region. In D. occidentalis, however, I found no cases of such extensive folding as he has shown.

Other structures that probably influenced Davidoff's conclusions, and are sometimes associated with the folding, are the cytoplasmic vacuoles near the germinative vesicle (Plate 3, Fig. 19). Oblique sectioning and a faint stain, such as the borax carmine used by Davidoff is liable to give, might combine to obscure the conditions so that the vacuole would appear to be part of the germinative vesicle. It may be that some of the nuclear evaginations seen by Davidoff were formed in this way; but, even if what he figures are actually evaginations from the germinative vesicle, it would not follow that they represented a normal condition. Thus the presence of the vacuoles (Fig. 19) just opposite to the *in*foldings of the membrane seem to show that both are artifacts due to some shrinking process.

These vacuoles, however, are certainly some of the structures which Davidoff has considered nuclear buds *separated* from the germinative vesicle. In younger ova, where all of the cytoplasm takes a much deeper stain, these vacuoles may have a stained periphery and thus resemble a nucleus very closely (Plate 3, Fig. 22, *vac.*).

The intravitelline bodies, from which Fol ('83), Sabatier ('84), and Roule ('85) have derived both follicle and test cells, and Pizon ('93, pp. 284–290) the test cells only, and the true nature of which Floderus first elucidated, are also present in Distaplia (Plate 1, Fig. 7; Plate 3, Figs. 22, 23; Plate 4, Fig. 28, *cp. ia'vt.*), though they are much smaller and occur less frequently than in Ciona. These bodies

are often, though not always, surrounded by a clear area, probably due to shrinkage, and especially when they are small, the combination looks very much like the nuclei with pale membranes and central chromatic spots figured by Davidoff ('89, Taf. 5, Figs. 6, 7). But the irregular occurrence of the clear area, the lack of a chromatic membrane in the later stages, and the absence of transitions between them and the test cell nuclei, show that they cannot be nuclei, and have nothing to do with the formation of the test cells.

b. Evidence for the Follicular Origin of the Test Cells.

The first indication of the formation of the test cells is found in the form and position of certain nuclei in the primitive follicle. Whereas at first all the nuclei of the primitive follicle are oval, when viewed in section, later one is occasionally encountered which is nearly spherical and projects some distance into the cytoplasm of the ovum (Plate 3, Fig. 13, *cl. fol.*). The space between the nucleus and the egg cytoplasm I do not suppose to be occupied by the hyaline cytoplasm of the forming test cell, as Floderus does in similar instances (p. 234), but consider it as due primarily to shrinkage. In some instances I believe that clear spaces thus formed were *partially* occupied by cytoplasm during life; but in these cases I think that there was nothing but the thinnest possible layer of cytoplasm between the follicle nucleus and the egg, for in my most satisfactory preparations of this stage, where the follicular cytoplasm is stained, none is usually encountered in this place (Plate III., Figs. 24, 25). Here it is seen that the stained cytoplasm of the follicle is not quite half as thick as the clear spaces in Figure 13, and that even here shrinkage vacuoles are occasionally present. They also show that in Distaplia no structureless membrane, or chorion, has as yet been secreted on the inner surface of the follicle, the line of demarcation between this surface and the ovum being merely the cell membrane of the follicle cells.

Figures 24 and 25 are from an ovum that is slightly older than that shown in Figure 13, and in the height of test-cell production. About half the follicle cells have rounded up, and either are on their way to become test cells, or have actually become such. A majority of these can be seen to be in cytoplasmic connection with the follicle. A perfect series of the stages in the process can easily be made out, from the enlarged follicle nucleus that is still in contact with the outer membrane of that layer, through cases of progressive migration of the nucleus and its accompanying cytoplasm into the ovum (Plate 3,

Fig 24, *cl. tst.*) to the condition where the test cell is entirely within the latter, but still connected by a narrow strand with the follicle (Plate 3, Fig. 24; Plate 4, Fig. 27, *cl. tst.'*), and finally to that where it is entirely disconnected from the follicle, and the chorion passes between the two (Fig. 27).

The way in which the test cells develop here is essentially that given by Morgan ('90) and Floderus (pp. 234, 235), but the evidence in the three cases differs slightly. Morgan has differentiated the boundaries between the adjacent follicle cells, which neither Floderus nor I have found, and has seen the cytoplasm of the forming test cell extending between the follicle cells quite to the peripheral membrane of the latter. Floderus finds both the follicle cell and the developing test cell within a clear space, which he considers hyaline cytoplasm. I find that in favorable instances the cytoplasm of the follicle and test cells stains, and the two are seen to be in connection. When, however, the cell boundary between them can be detected (Fig. 25), the test cell cannot be seen to extend quite to the periphery of the follicle, but lies on its central surface. In this respect Distaplia differs from the forms studied by Morgan, for the first step in the formation of the test cell in Distaplia seems to be a displacement of the whole follicle cell towards the inner surface, and only subsequently does its separation take place.

Julin (p. 123), in describing the ovogenesis of Styelopsis, says that there is an almost simultaneous mitotic division of all the follicle cells, and when the products of this division come to rest, they are arranged in two layers, the inner of which consists of test cells. Caullery ('94, p. 600) agrees with Julin in deriving the test cells from the follicle by mitosis. I have never seen any mitotic figures in the follicle or test cells of Distaplia occidentalis, but do not doubt that they occur. It is likely, however, that in this species the process is not accomplished as Julin describes it, for after the mitosis there must be a differentiation taking place, by means of which the nuclei of the prospective test cells become larger and more spherical than the remaining follicle nuclei.

Shortly after the origin of most of the test cells, the chorion is secreted between these cells and the follicle (Plate 4, Fig. 27); even this, however, does not destroy the cytoplasmic connections of all the test cells, and occasionally the union can be seen at much later stages. With the formation of the chorion, the differentiation of the primitive follicle into secondary follicle and test cells appears to be complete,

and the large subsequent increase in the numbers of the latter must be due to their division. There is no reason to doubt that they divide by mitosis, for at this stage they have a perfectly healthly appearance, and in fact Davidoff (pp. 131–132) has described and figured mitoses of both test and follicle cells in D. magnilarva.

c. Degeneration of the Test Cells.

The degeneration of the test cells in Distaplia is a much simpler process than that occurring in some ascidians, consisting, as Davidoff has shown, in the vacuolation of the cytoplasm. The cellular nature of the product is always evident, as the distinctive character of the nucleus is never lost, though this organ is forced into a peripheral position and changes its appearance slightly. This degenerative vacuolation begins in our species at about the same time as the yolk formation (Plate **4**, Fig. 28) and is completed (Plate **5**, Fig. 30) before the last changes in the yolk occur. Figure 29 (Plate **4**) shows a few test cells in which the process is just beginning. At this stage the test cells are often numerous enough to form a double row about the periphery of the ovum (Fig. 28), but sometimes they form only a single row. As the ovum passes through the oviduct into the pouch, it seems to contract somewhat, forcing the test cells out, and the yolk bodies between which they were formerly situated become arranged so as to form a smooth surface at the periphery of the ovum (Plate **5**, Fig. 31). However, in spite of this tendency, there is occasionally enough pressure exerted upon the test cells to force them partly into the yolk again.

Up to this time the nucleus has changed little if any, but from now on further changes in the test cells are almost entirely confined to this organ. During the growth of the embryo, the nucleus loses most of its chromatin, its membrane only staining, and becomes compressed between the cell membrane and adjacent vacuoles (Plate **5**, Figs. 32, 34).

d. Fate and Function of the Test Cells.

Among the early investigators the belief that the test of the larval ascidian was formed by the test cells was universal, and it was on this account that Kupffer ('70, p. 122) applied this name to them. In 1872, however, Herting ('72) showed that the test first appeared as a thin layer next to the ectoderm of the larva and entirely within the so-called test cells, which took no part in the process. This view has been gen-

erally accepted from that time; but quite recently a vigorous supporter of the older theory has been found in Salensky ('92, '94, pp. 441–449, '95, pp. 618–621).

This author, finding that in Salpa and Pyrosoma cells which are derivative of the follicle take a large part in the formation of the embryo, turns to the compound ascidians expecting to find there the beginnings of the same process. Accordingly, he makes out that in the compound ascidians the test cells, or kalymmocytes, as he calls them, have a function to perform during the life of the embryo, though this differs in different cases. In representative species of the genera Fragarium, Amarœcium, and Circinalium he found a placenta attaching the embryo to the wall of the cloaca, and the fœtal part of this structure was derived from transformed test cells. In Distaplia magnilarva, Diplosoma, and Didemnum these cells have the function of forming the test of the larva, the whole of it being derived from the test cells in Diplosoma, but only a part of it in the other two genera.

In Distaplia, which is the only genus in which this process need concern us, Salensky finds that at the time when the test is forming, the test cells flatten out against the ectoderm of the larva so that in places they form continuous layers. These he thinks secrete the cellulose matrix ('92, pp. 113–114) and then metamorphose into the cells lining the lacunar spaces with which the test is honey-combed. The test, he thinks, continues to grow in the same way, having layer after layer of test cells added to its outer surface. Caullery ('94, p. 600), the only other author who has studied this subject since the publication of Salenky's first article ('92), comes to the conclusion that the test cells take no part in the formation of the test, but does not give any evidence.

In examining Distaplia occidentalis, an appearance was quite frequently noted that looked very much as if there were unmodified test cells within the test itself. But when examined more carefully, the substance within which the test cells were contained was found at stages before there was any test being formed, and even before the egg had segmented. It was also found within the cavities of some of the older embryos, and was then concentrated on the same side of all the cavities. If in these cases the substance was situated on the left side of the cavities, then that on the outside of the embryo was always on the left side and vice versa. Thus there was every reason to believe that the substance surrounding the test cells was a coagulum that had been thrown down by the fixing reagents, and had reached its character-

istic arrangement by means of gravity. From Salensky's ('92, p. 113) statement that in an early stage the changing test cells are separated from one another by a homogeneous substance that looks very much like test matrix, I have thought that he might possibly have had some more refined form of a similar coagulum before him; but I cannot for a moment suppose that the true nature of a state of affairs such as exists in my preparations would have escaped him.

The flattening out of the test cells against the ectoderm and test mentioned by Salensky, I find to be due to pressure from without. Wherever the inner follicular epithelium is close to the embryo, as on its sides, for instance, there the test cells are much flattened against the surface (Plate 5, Figs. 32, 34, *cl. tst.*); but where the epithelium is lifted from the surface, as in the triangular space it leaves when passing over the tail, the great majority of the test cells are spherical. Occasionally, however, oval ones are found here with their sides next to the ectoderm or test, but in such cases the extreme flattening that occurs on the sides has never been seen. Moreover, in reviewing quite a number of series I have been unable to discover any transitions between the test cells and cells within the test, or any cases in which the peripheral layer of the test matrix was not perfectly distinct from the superimposed test cells. Thus no evidence at all has been found that is favorable to Salensky's view of the test formation.

So much for the interpretation of Salensky's results. Concerning the actual method of test development I must say that I think it can be shown that the test in Distaplia is formed in such a way that the test cells cannot be instrumental in producing it.

The first tunicin, or animal cellulose, that is laid down is that forming the tail fin, which is quite well developed at a time when no other tunicin can be detected either on the tail or the body of the embryo. Now the test matrix of the fin has no cells within its substance, and so must have been formed by the activity of cells that are outside of it. As it is attached to the ectoderm on one of its three sides, and as the test cells are in contact with only about half of the surface of the other two, and are usually not pressed against it, and as these cells are in no way different from any of the other test cells, there is very little ground for believing the test matrix of the tail to have been secreted by the test cells.

On the body of the embryo an examination of the early stages shows that here the whole thickness of the test is quite small, much less than the thickness of a test cell, even though the latter is pressed against the

test by the follicular epithelium (Plate **5**, Fig. 32, *tst.*). In spite of this fact, however, the outer layer of the test matrix is quite thin, and under it the beginnings of the first lacunæ (*lac.*) are seen. Occasionally we see cells (Fig. 32, *cl. ms'drm.*) within the lacunæ, but these have none of the characters of the test cell, whereas they do resemble some of the mesenchyme elements very closely. It seems hardly possible, then, that the test cells can have had anything to do with forming this structure. Moreover, in following the series through ten sections, 6 μ thick, not another cell is found within the test of this region. It is seen therefore that the first tunicin and some of the first lacunæ are formed from the ectoderm, not only without the intervention of the test cells, but also without the aid of *any* cells lying within the test substance.

With further increase in the thickness of the test the lacunæ increase in size, and many mesoderm cells wander into them, and into the matrix between them (Plate **5**, Figs. 33, 34, *cl. ms'drm.*). These cells often flatten out against the lacunar wall, and line it for a variable distance, but in the test that is about as thick as the test cells; this condition is rarer than at a later period, when there are several layers of lacunæ.

During the whole of the development, up to the time when the larva is extruded, the test cells are often closely pressed against the test; but there appears to be no marked decrease in the number of these elements, though, of course, on account of the greater size of the embryo, in the later stages they become more widely separated. In this species, then, the evidence points strongly to the conclusion that the test cells have nothing whatever to do with the formation of the test, but that the matrix of the latter, together with its lacunæ, is formed from the ectoderm, and that its cells are mesodermic elements that have subsequently wandered in.

What, then, is the function of the test cells in Distaplia, if they have nothing to do with the formation of the test, and what is the function of these cells in general? Many authors are not satisfied with their rôle in nourishing the ovum, but think that they must be functionally active as long as they remain near the growing embryo. Thus, Salensky ('92, pp. 109–110) says: "Es wurde vielmehr still schweigend angenommen, die Testazellen hätten keine Bedeutung bei der Bildung des Embryos der Ascidien. Ein solcher Schluss war für mich von Anfang an nicht ganz befriedigend." Floderus, after a careful discussion of the literature, so far fails to realize the true nature of these cells that he says (p. 244): "Ich halte es für wahrscheinlich, dass die Testazellen eine Art von rudimentären Bildungen sind, welche nunmehr eine un-

bedeutende Rolle spielen, allein einstweilen dürfte man die Frage nach ihrer eigentlichen Function und ihrer richtigen Deutung gewissermassen als eine offene bezeichnen können."

In considering the function of the test cells, it should always be borne in mind that they are *follicle cells* derived from other follicle cells, and as such their mission is to convey nourishment to the ovum. The fact that they lie under the chorion, and imbedded in the cytoplasm of the ovum, does not remove them from this category, but merely puts them in a more favorable position for conveying food to the egg cell. Starting as normal and vigorous cells in the follicular epithelium, their activity is so great that degenerative changes in the shape of vacuolation appear in them, while the follicle cells proper still retain their normal appearance. The early occurrence and complexity of these processes offer the best possible evidence for the intensity of the activity. It is not surprising, therefore, and I think we have no right, *a priori*, to expect that cells which have worked so hard that they have lost their vitality — cells in which degenerative changes have set in — should become further involved in the developmental processes of the embryo.

3. Secondary Follicular Epithelium.

For some time after its complete differentiation this epithelium is comparatively thin with flattened nuclei (Plate 4, Fig. 27). Soon, however, as the cells multiply, the epithelium thickens, and the nuclei assume a spherical shape (Plate 3, Figs. 26, 15, *e'th. fol.*). The increase in cells continues, so that soon they must arrange themselves in two layers (Plate 4, Fig. 28). At about this stage morphological differentiation sets in, and the inner nuclei fail to take the stain strongly, thus becoming somewhat paler than before (Fig. 28, *nl. e'th. fol. i.*). All cell multiplication now seems to cease, for with the subsequent growth of the ovum the thickness of the follicle decreases, probably on account of stretching (Plate 5, Fig. 30). But in spite of this thinning out the follicle does not become one cell thick, as Davidoff maintains (p. 136). On the contrary, there are two quite distinct kinds of nuclei (Fig. 30), and it is probable that at this stage the inner and outer follicular epithelia are differentiated, but so closely pressed together that the only indication of the differentiation consists in the nuclei.

4. Inner Follicular Epithelium.

It must be confessed that while the egg is still in the ovary the nuclei of this layer are hard to find, and do not appear to be numerous. More-

over, in the latest ovarian stages, where the follicle is even thinner than in Figure 30, they are still less apparent. But later developments show conclusively that we here have to do with the nuclei of the inner follicular layer; for when the egg has left the ovary it is covered by only a thin pavement epithelium with pale nuclei at rather distant intervals (Plate 5, Fig. 31, *e'th. fol. i.*), while all the darker nuclei of the outer follicle remain behind in the corpus luteum (Plate 1, Fig. 5; Plate 6, Fig. 47). This inner epithelium persists, and covers the embryo during the whole of its life within the colony, and becomes so stretched in the later stages that even the nuclei are obliterated. It is thus seen that, contrary to what Davidoff believed (p. 138), there is in Distaplia a splitting of the secondary follicle into an outer and an inner epithelium, which is actually accomplished only when the egg leaves the ovary, though the two layers are probably, and their nuclei certainly, differentiated some time before. Thus, in Distaplia, the results of van Beneden et Julin on the origin and fate of these two epithelia are completely confirmed.

5. Outer Follicular Epithelium and Corpus Luteum.

As has already been said, one of the distinguishing features of this layer is the comparatively deep stain that its nuclei take. Another characteristic, which is just beginning to appear in Figure 30 (Plate 5), but is much more distinct later, is that part of the cytoplasm also takes a very deep stain. Tangential sections through the follicle of ova that have reached the maximum size, about 300 μ, show this stained cytoplasm situated in irregular patches around a central nucleus. In sections of the newly formed corpus luteum (Plate 6, Fig. 47) this cytoplasm comes out very distinctly.

The corpora lutea (Fig. 5) are often conspicuous features of the Distaplia ovary, as they are at first about 80 μ in diameter, and remnants of them persist until an embryo of forty or fifty cells is developed from the ovum formerly contained within them. When first formed, this structure is a thick walled vesicle with a rather small cavity opening into the lumen of the ovary. Almost the whole thickness of the wall is composed of the cells of the outer follicle, which are pressed together and elongated radially. Figure 47, which represents part of the wall of a corpus luteum which the hind end of the ovum has just left, shows most of the deeply stained cytoplasm of each cell located between its nucleus and the periphery of the organ. Peripherally, this

cytoplasm seems to be attached to the hyaline membrane (*mb. prp.*) surrounding the whole structure. This membrane is nothing but a local differentiation of a similar covering of the ovary and all its products, the consideration of which has been postponed until now.

Distaplia seems to have a special aptitude for the secretion of structureless membranes, for they cover the surfaces of nearly all the visceral organs. In the case of the ovary and its products, the membrane can usually be demonstrated with ease. Both the connective-tissue cells and the epithelium of the ovary itself seem to participate in the secretion. Origin from the first source is to be inferred both from the frequency with which strands of connective tissue are seen to join and extend along the wall of the ovary (Plate 1, Fig. 6, *fun. con't. tis.*); and also from the fact that often the connective tissue cells themselves are found on the wall of the ovary. That the membrane is also in part secreted by the cells of the ovary is proved by its general occurrence, but principally by the fact that on the periphery of the stalk tissue, where we have the greatest number of cells per unit of area, it is exceptionally developed, being much thicker than at other places (Fig. 5, *mb. prp.*). It occurs also on the surface of the follicles, varying much in thickness, but being in general best developed on those that are about half grown. On the surface of the oldest follicles are found here and there very flat nuclei that are probably derived from wandering cells almost imbedded in this membrane. It is probably nuclei of this kind, and the structureless membrane containing them, that Julin considers to be the remnant of the limiting epithelium of the ovary surrounding the follicle; for he says that this limiting epithelium has gradually become hyaline.

On the newly formed corpus luteum this basement membrane (Plate 6, Fig. 47, *mb. prp.*) is thicker and more conspicuous than on any other part of the ovary, except some of the stalk tissue, and it is of special importance in attempting to explain how the ovum is pressed through the ovary into the oviduct. This pressure cannot be due to muscles, as there are none in the abdomen except those of the heart. Nor can an increase of blood pressure in the whole abdomen, due either to long-continued beating of the heart in one direction, or to the contraction of thoracic muscles, be invoked in explanation. For while this might burst the follicle by pressing it against the ectoderm and modifying its spherical shape, it would also tend to press the oviduct out flat against the ectoderm, and so could hardly be effective in moving the ovum into the oviduct. The only other available force

is one resident within the follicle of the ovum, and this might lie either in the outer follicular epithelium or the membrana propria. I think that at first both of these structures are the active agents; but the irregular crowding together of the follicle cells in the corpus luteum seems to show that, during the later stages of the process, at any rate, the cells have no active function. Finally, then, by this method of exclusion, we come to the membrana propria as par excellence the active agent in forcing the ovum into the oviduct, and find that there is considerable direct evidence for this view. In the first place, both follicular epithelium and membrana propria are presumably under tension in the largest eggs, being formed by the stretching of structures that were formerly thicker and of less area. Secondly, the membrana propria of the corpus luteum is perfectly homogeneous and quite thick; and it is very improbable that a membrane which was formerly very much thinner, and covered an area about fourteen times its present extent could assume this condition unless it had been under tension, upon the removal of which it could contract into its present shape. Thirdly, it has the appearance of elastic substance, being thrown into the folds characteristic of such material. These folds, however, may in part be due to the irregular pull exerted on the membrane by some of the follicle cells attached to it. When once the ovum is started on its course up the oviduct, the continuation of its progress seems to be effected by the contractions of the oviducal epithelium, but the main force in the initiation of the process seems to be the elastic tension of the membrana propria of the follicle.

As soon as the ovum has entirely passed out of the ovary, the corpus luteum begins to degenerate. The first change consists in the dissociation of the deeply stained cytoplasm formerly associated with the nuclei (Fig. 5). Strands of this material are still encountered, but the characteristic arrangement is lost. At about the same time the nuclei themselves appear paler, and the wandering mesoderm cells on the surface of the structure are flattened against it, and together with the membrana propria form an investing capsule (Fig. 5). Next comes the constriction of the stalk of the corpus luteum, by means of which it is separated from the stalk tissue. During this process the lumen may or may not disappear, but after the distinct epithelial connection with the stalk tissue is lost, the corpus luteum remains connected therewith for some time by irregular strands of cytoplasm or connective tissue.

Within the isolated corpus luteum many vesicles are formed contain-

ing nuclei in various stages of chromatolytic degeneration, while outside the vesicles are other nuclei, which, though staining faintly, appear to be in a healthy condition. It seems, then, that some of the cells of the corpus luteum retain their vitality and digest their neighbors, for the functional nuclei are so numerous as to make it improbable that they have all penetrated the thick membrana propria from without.

Cases have been encountered where this cutting off from the ovary has already taken place when the ovum that left the corpus luteum is in the two or four cell stage. On the other hand, the same condition has been found when the youngest embryo in the incubatory pouch contained about sixteen cells. From this time on, the remnant of the corpus luteum becomes smaller and smaller, still remaining a compact mass; but finally, when the ovum has divided into about sixty cells, it disintegrates and disappears.

6. Observations on Styela montereyensis.

In this species the development of the follicular membranes progresses in much the same way as in Distaplia, but it is more difficult to follow. The presence of a separate outer follicular epithelium, however, is easily seen at a comparatively early stage; but, as is usual with the simple ascidians, this epithelium is much thinner than the inner follicle.

The most interesting events in the life of the follicle of this species are the degenerative changes which take place in the test cells, and those of the inner follicular epithelium. In the latter the process is comparatively simple, and consists only in the development of a large refractive body in the cytoplasm of the cell. It is first seen at a stage when the yolk is fully formed, and the maximum size of the ovum is nearly reached, and appears as a small speck in the cytoplasm, so close to the nucleus that it may have been extruded from the latter. It rapidly increases in size, until, just before the egg leaves the ovary, it is nearly as large as the nucleus of the cell and is surrounded by a lighter area, which may, however, be due to shrinkage. Together with this area it is about the size of the nucleus, which now occupies an excentric position. It does not take nuclear stains well, but when a safranin, gentian violet, and orange triple stain is used, it is colored an opaque brown, that makes it the most conspicuous object in the ovary. This body can have no reference to any future function of the

cell, for, very shortly after the egg enters the water, into which it is extruded, all of the follicle cells are lost, and the egg is covered only by the chorion, with a few cellular fragments adhering to its outer surface. It seems rather to be some product of the intense metabolism of the cell, which the latter is attempting to get rid of, or to deposit in some innocuous form.

The degenerative changes in the test cells are much more complex, ending in a product that looks like Figure 46 (Plate 5) when stained with a nuclear stain, and like Figures 42 and 43 (Plate 5) when overstained in safranin. The latter stain brings out the essential structure more clearly, and by means of it we see that the test cell has resolved itself into a number of vesicles (*vs.*), each one of which contains a central refractive, faintly staining corpuscle (*cp. c.*). The number of vesicles varies from seven or eight to twice that number. They are remarkably uniform in size, and when well stained it is only very rarely that one is encountered which lacks the corpuscle, though it often happens that nuclear stains do not bring out the corpuscles at all satisfactorily. At the centre of the cell there is accumulated another substance, which sends out thin lamellæ between the vesicles. In hæmatoxylin preparations (Plate 5, Figs. 44–46), the central substance appears homogeneous or finely granular, but safranin demonstrates within it quite a number of very deeply staining bodies that sometimes occupy almost the whole central space (Fig. 43). Of the nucleus, not a trace can be seen, for, as will be shown presently, the central bodies just mentioned are not composed of nuclear material.

The first step in the formation of these complex test cells begins early, not very long after the formation of the test cells themselves, and before the yolk granules appear. The first change noticed is an irregular vacuolation of the cytoplasm (Plate 5, Figs. 35, 36). Within many of these vacuoles are seen bodies (*cp. ia'vac.*) which look much like the central corpuscles of the vesicles of the final product, so that it is most natural to suppose that the vesicles are developed from the vacuoles of the earlier stage. But the conditions subsequently encountered do not bear out this supposition.

The next step consists in further vacuolation, which is still more irregular. Some of these vacuoles are much larger than any of the vesicles of the final stage (Plate 5, Fig. 37), and occasionally three-quarters of the cell will be taken up by an immense vacuole crowding the smaller ones and their central bodies to one end. Furthermore, nearly half of the vacuoles have no stained bodies within them,

and these bodies themselves have increased in size, so that instead of being comparable to the central corpuscle, they are nearer the size of the entire vesicle of the final stage (Plate 5, Figs. 37, 41). At this stage there are two kinds of stained bodies, — those within the vacuoles (*cp. ia'vac.*) and those included in the lamellæ between the vacuoles (*cp. ia'll.*). Safranin (Fig. 37) does not allow one to distinguish between these, but the triple stain of safranin, gentian violet, and orange does, giving the intravacuolar bodies a light yellow, and the intralamellar ones a deep purple color. At a slightly earlier stage the intravacuolar bodies also take the purple stain, so that it may be that these two structures have a common origin. The nucleus takes part in none of these changes. It is occasionally seen in safranin preparations (Fig. 38, *nl.*), and hæmatoxylin shows it to be practically unchanged, and universally present at considerably later periods (Plate 5, Fig. 44).

The next and most important stage is that in which some of the intravacuolar bodies have acquired a central more deeply stained corpuscle. Their size is about the same as before, but they stain more faintly (Fig. 38). In the next stage (Plate 5, Figs. 39, 40) all of these bodies have developed a central corpuscle; they now take a still fainter stain, and have approximated still more closely in size to the vesicles of the final stage. In fact they resemble the latter so closely that most of the transitional stages from one to the other are found among the test cells of a single ovum. As these bodies acquire their final dimensions, they assume a peripheral position, crowding the remains of the intravacuolar cytoplasm towards the centre, in which are contained the deeply stained intralamellar bodies that treatment with safranin makes so prominent (Plate 5, Figs. 42, 43).

The nucleus, which from the start of the process has a peripheral position, persists almost unchanged until the vesicles have become thoroughly established (Fig. 44). It is still oval, but stains more faintly than before. Later still, while the vesicles remain entirely unchanged, the nucleus slowly degenerates, becoming at first paler, and distorted by the pressure of the vesicles (Fig. 45), and then refusing to take the stain altogether, but persisting as an irregular peripheral refractive patch (Fig. 42, *nl.*), which finally seems to vanish entirely (Fig. 46).

This process, like that in the follicle cells, has no prospective meaning, for the test cells take no part in the development of the embryo. In life they have a decided yellow color in this species, while the

yolk is green, so that, in the living embryo, they can be detected easily, floating in the space between the embryo and the chorion, and are seen to be left behind at the time of hatching. The process must rather be considered as entirely degenerative, perhaps a futile attempt to conform to the changed conditions and protect the cell from the disastrous results of its active metabolism, by means of which the ovum is nourished. It seems well to emphasize the intensity of this metabolism.

V. Ovum.

1. Cytoplasm.

As is usual in ascidians, the cytoplasm of the young Distaplia ova is filled with rather large granules that take a nuclear stain with avidity (Plate 1, Fig. 4; Plate 3, Figs. 21, 22), but while the test cells are being formed the stain becomes fainter and fainter, until it almost vanishes. Together with the decrease in stainability, there is a decrease in the size of the granules (Plate 3, Figs. 23, 13), until at last they become so inconspicuous as to be negligible quantities (Plate 4, Fig. 27). In later stages again they appear to become more marked, but it is difficult to correlate the changes with the size of the ova, for there is so much individual variation. It is clear, however, that after the cytoplasm has ceased to stain deeply, good preservation will often enable one to detect a cytoplasmic network, the nodal points of which give the appearance of granules (Plate 3, Fig. 26; Plate 4, Fig. 28). This condition comes out more distinctly in the later stages figured, but indications of the same thing are seen earlier, and the reticulum probably exists even where it is obscured by the deep stain. The network persists up to the time of the formation of the yolk bodies, and is present in the central part of the ovum when the periphery already contains much yolk (Fig. 28).

In Distaplia magnilarva Davidoff ('89, p. 155) did not find any stages in the process of yolk formation, and hence concludes that the yolk bodies are formed simultaneously throughout the whole extent of the ovum. In our species, however, stages are often encountered in which the yolk bodies are present at the periphery only (Fig. 28). Here the largest yolk bodies are scattered amongst the test cells, and as the germinative vesicle is approached they become progressively smaller, until near the centre there is a gradual transition into the cytoplasmic reticulum. However, where the yolk is forming the

whole of the cytoplasm seems to be converted into that substance, for the intervals between the yolk bodies are not occupied by the cytoplasmic reticulum, — which at this stage takes a distinct though faint stain, — but by a confused mass of substance, all of which has the characteristic hyaline appearance of the yolk bodies, in which, however, no structures of definite shape have been formed. After the yolk bodies are fully established, the absence of undifferentiated cytoplasm between them is still more marked, for, like Davidoff, I have been unable to discover trace of any other substance between them (Plate **5**, Figs. 30, 31; Plate **6**, Fig. 50). When fully formed, the yolk bodies are of very large size for ascidian material, and of various shapes. Those apparently first formed are oval or spherical, while those of later development are angular and occupy the spaces left by the first ones.

Yolk formation begins when the ovum is about half grown (150 μ in diameter), and all of the cytoplasm is transformed into yolk some time before the maximum size (about 300 μ) is reached. During the growing period that follows the earliest stage at which all the cytoplasm is converted into yolk, there is a little clump of yolk around the germinative vesicle which has but indistinctly broken up into yolk bodies (Plate **6**, Fig. 49), and it may be from the periphery of this clump that the new yolk bodies producing the increase in size are formed. Ova possessing this clump of undivided yolk average about 200 μ in diameter.

Later this clump becomes completely broken up into yolk bodies (Fig. 50). But none of these are spherical, as is usually the case in other regions, all being more or less elongated in a radial direction. It is probable that growth continues even after this central yolk has broken up, for the range in size of ova in this condition is from 230 μ to 326 μ. It is impossible to determine the diameter of the mature ovum, because, as soon as it begins to leave the ovary, it is immediately deformed, and even in the pouch it is oval instead of spherical. But it is not probable that the mature ovum would exhibit so much variability.

2. Germinative Vesicle.

The chromatin in the young germinative vesicle is usually situated next to the membrane, while in the centre no structural elements of any kind can be detected (Plate **1**, Fig. 4, *ov'go.*). The chromatic granules are sometimes of uniform size (Fig. 4, *ov'go.*), but usually there is one which is much larger than the rest, and destined to become

the nucleolus (Fig. 1, *ov'go.*). At this period all of the granules, including the prospective nucleolus, are differentiated similarly — taking a bluish tint — with the methyl green and acid fuchsin double stain. Soon achromatic fibres make their appearance. At first mainly peripheral, and inserted on the chromatic granules and the membrane, they soon come to traverse the centre of the vesicle. It is rarely, however, that chromatin is found upon them (Plate **1**, Fig. 3, *ov'go.*). At the same time that the achromatic network becomes differentiated, the largest chromatin granule increases in size, and becomes more nearly spherical and not so closely approximated to the membrane. Although it still takes the same stain as the other granules, the disparity in size and its regular shape justify us in calling it the nucleolus. Its later development will be discussed in a separate section.

The further changes that occur in the growth of the rest of the germinative vesicle consist entirely in the elaboration of the achromatic network and the chromatin granules upon it. The achromatic fibres are not always seen, especially in the later stages, but staining in iron hæmatoxylin brings out the chromatic elements very distinctly (Plate **3**, Fig. 13; Plate **4**, Fig. 27; Plate **6**, Fig. 48). The latter form a reticulum extending through all parts of the germinative vesicle, the complexity of the structure increasing with the size of the vesicle, and culminating in the condition shown in Figure 48 (Plate **6**).

In addition to the reticulum, many isolated granules are found throughout the vesicle and especially on its membrane. While all these granules when stained with iron hæmatoxylin are very deeply colored, and resemble closely the chromatic structures described for other eggs, still, strictly speaking, we are not justified in calling them chromatin; for chromatin is that substance which takes the chromatic or basic aniline stain when treated with a combination such as methyl green and acid fuchsin. Malfatti ('91), in testing the electivity of this stain for known chemical substances, found that free nucleic acid was stained pure green; nucleins, which contain less phosphorus, were colored blue, while substances with still less phosphorus took the red stain. Thus, as nucleic acid and the nucleins are derived from the nucleus, it becomes highly probable that chromatin is made up of these substances.

Now, when the germinative vesicle of this stage is treated with the stain mentioned, the network that is so distinct in hæmatoxylin preparations is colored red, and the only substance staining green is con-

tained within the nucleolus, which will be considered later. During the growth of the nuclear reticulum, then, there appears to be a concentration of nucleic acid within the nucleolus, while the reticulum itself and all the other granules within the vesicle suffer a decrease in their percentage of phosphorus.

The condition of the germinative vesicle shown in Figure 48 (Plate 6) is found in ova about 150 μ in diameter, in which the yolk bodies are just beginning to be formed. During all of the subsequent growth of the ovum, while it doubles its diameter, the germinative vesicle *decreases* in size, usually diminishing to a little less than half its former diameter (see Table, p. 100). During this shrinking, the vesicle exhibits the wavy and stellate outline characteristic of the stages preceding the maturation of ova (Plate 6, Figs. 49, 50); here, however, the shrivelling is associated not with maturation but with yolk formation.

This shrinking of the germinative vesicle and the formation of the maturation nucleus from it have been studied by Davidoff (pp. 156–159), according to whom the process is very complex and quite unique. First, the membrane disappears, and the major portion of the contents of the germinative vesicle forms the "ergoplasma," or active cytoplasm, which gradually becomes disseminated between the yolk bodies. The nucleolus is the only structural element that remains behind, and it undergoes the most complex modifications. It takes a deep stain, and becomes shrivelled so that its outline is irregular and at times stellate. He says he believes that there is an actual decrease in the volume of the nucleolus accompanying these morphological changes, but his figures do not bear him out in that statement. The next change is, that within the shrivelled nucleolus small chromatic granules are differentiated, which soon aggregate to form a compact chromatic body. This itself subsequently becomes vesicular and contains a central granule. A few of the chromatic granules do not take part in the formation of the compact chromatic body, but initiate the formation of a dense chromatic network, with which the whole nucleolus becomes filled. At the same time a membrane is distinctly differentiated about the periphery of the nucleolus and we have developed from it a nucleus which is neither germinative vesicle nor nucleolus, but the maturation nucleus or "Polkern."

In Distaplia occidentalis, most of the stages discussed by Davidoff are abundantly represented; but I must differ from him in their interpretation and in the order of their sequence. I will first describe the process as I conceive it to be, and then compare my results with those of Davidoff.

At the time when the first yolk is being formed, the germinative vesicle has a full rounded outline and its maximum diameter of about 45 μ (Plate 6, Fig 48). As the formation of the yolk continues, and the ovum grows, the germinative vesicle shrinks, its membrane becomes wavy, and before all of the cytoplasmic reticulum has become transformed into yolk, the vesicle has shrunk to a diameter of about 30 or 35 μ. In the next stage (Fig. 49), the yolk extends up to the membrane of the germinative vesicle, but the central yolk clump is not distinctly separated into the yolk elements. In ova of this stage the germinative vesicle has an average diameter of about 25 μ. Finally (Plate 6, Fig. 50), when the ovum is larger still and the central clump of undivided yolk has broken up, the germinative vesicle has shrivelled to a diameter of about 20 μ (see Table, p. 100).

During the whole of this process, the methyl green and acid fuchsin stain differentiates the nucleolus within the shrivelling germinative vesicle. With some other stains however it cannot be seen, for the germinative vesicle is so deeply stained that no structure at all can be distinguished within it. Iron hæmatoxylin usually has this effect, and the stain is so intense that excessive decoloration does not differentiate a vesicle, but works from the periphery inward, removing all the stain from around the edges, while in the centre it persists with undiminished intensity. Occasionally, however, colonies are encountered in which the stain does not work this way, but differentiates a vesicle, and in these cases (Plate 6, Figs. 49, 50) the nucleolus and the remains of the reticulum are plainly seen.

In order to make perfectly sure that the sequence noted above is correct, I established four classes, based upon the sequence mentioned, and then measured all the eggs in these stages contained in several series, placing each egg in the class to which it belonged according to the structure of the yolk, and germinative vesicle. If my sequence is correct, by this means I should get a progressive increase in the size of the ova in the successive classes, and when passing from one class to the next, and corresponding to this a diminution in the size of the germinative vesicle. This condition is actually what I did obtain.

The classes were: —

1. Yolk bodies formed at the periphery only. Germinative vesicle with a full rounded outline.

2. Central part of the reticular cytoplasm not yet differentiated into yolk. Germinative vesicle with a wavy outline.

3. Yolk extends from the periphery to the germinative vesicle, but

the central clump has not yet broken up into yolk bodies. Germinative vesicle stellate.

4. Central yolk clump has broken up into yolk bodies. Germinative vesicle stellate.

Out of 48 eggs measured, the various sizes, expressed in micra (μ), were distributed as follows : —

Class.	No. of Ova in each Class.	Smallest Ovum.		Largest Ovum.		Average.	
		Size of Ovum.	Dimensions of Germ. Ves.	Size of Ovum.	Dimensions of Germ. Ves.	Size of Ovum.	Dimensions of Germ. Ves.
1	10	133	56 × 50	173	52 × 42	151	47 × 37
2	6	134	46 × 26	191	33 × 25	161	36 × 28
3	15	170	31 × 30	243	28 × 15	205	28 × 20
4	17	230	28 × 25	326	20 × 19	287	23 × 17
Total	48						

From this table, it is seen that the classes established on morphological grounds contain groups of similar ova, and hence represent the actual sequence of events. The column of averages especially shows well how the germinative vesicle diminishes with the growth of the ovum.

Among the fifty-two ova examined, four exceptions were encountered that would not fit well into any of the classes. Two of these were small ova, about on the transition line between the second and third classes, but in which the cytoplasmic conditions were very indistinct. The other two belong to an entirely different category, and from their rare occurrence in my early series, from which the above data were taken, as well as in the later series, they must be considered abnormal. In them the yolk bodies were completely differentiated in all regions, but the contents of the germinative vesicle was finely granular and not separated by a membrane from the yolk.

From this account it follows that the shrinking of the germinative vesicle is a continuous process, and much simpler than Davidoff considered it. In attempting to interpret his results in the light of these investigations, there is but one point that offers any obstacle. That is his initial stage in the shrinking, in which the membrane of the vesicle disappears and its contents penetrate between the neighboring

yolk bodies; while the nucleolus remains behind (Davidoff's Figures 18, 19, Taf. V.). It will be seen, however, that this condition is exactly what I have found in the two abnormal ova mentioned above. It may be that this appearance is more common in the species studied by Davidoff than in the one with which I am familiar, and this stage may possibly have some place in the normal series of events in that species, but the close similarity of his other stages to mine does not favor this view.

Davidoff's ergoplasm that in some stages surrounds what he takes to be the nucleolus, but finally penetrates between all the yolk bodies, is, I think, the central clump of yolk surrounding the germinative vesicle in class three. When stained faintly, it sometimes looks slightly granular. But so far as the penetration of the yolk by this substance is concerned, I cannot confirm his results; for in the later stages, as in the earlier, I find no interstitial substance whatever between the yolk bodies.

The body that Davidoff takes to be the nucleolus in the later stages is undoubtedly the shrunken germinative vesicle. In size, shape, and in all other respects, the two objects are entirely similar. Curiously enough Davidoff follows quite a number of these stages backwards, in spite of the fact that he thinks that his "nucleolus" is *shrinking*, and that his figures show the size of that structure to be increasing in the successive stages. The central body which he finds developed within his nucleolus is nothing but the true nucleolus of the vesicle, which I have traced through all the stages, but which would often be obscured by the stain he used. His main trouble consists in not having had before him any of the first stages in the shrinking of the vesicle when its membrane and nucleolus are easily seen. On this account also he missed the entire process of the transformation of the cytoplasmic reticulum into yolk, which occurs at the same period.

That this shrivelling of the germinative vesicle is closely associated with the formation of the yolk, is suggested by the synchronism of the two events, and it is interesting to note that the intense activity of the nucleus, of which this shrinking is probably the result, begins shortly after degenerative changes have commenced in the test cells. It seems, then, that the test cells are particularly active in conveying nourishment to the ovum in the early stages, whereas the nucleus exerts its principal activity in the later stages in converting this material into yolk.

3. Nucleolus.

The nucleolus was left at the time when it had just assumed a nearly spherical shape, but was still attached to the membrane of the germinal vesicle. It progressively assumes a more central position, but remains attached to the membrane for a considerable period by means of a stalk (Plate 3, Figs. 20–22). This stalk is at first quite large, but becomes smaller as the nucleolus increases in size, and finally disappears altogether, never being encountered during the later stages. It cannot be seen in all the young vesicles, and would of course be invisible except in a profile view; but from the generality of its occurrence I believe it is a normal structure serving to attach the nucleolus to the membrane from which it has been derived. Floderus (pp. 214–215) has described nucleoli with similar projections attached to them, and believes that he has in them stages in the formation of "nebennucleoli," but the fact that the largest stalks are attached to the smallest nucleoli, which is also brought out by his figures (Taf. X., Figs. 19, 20, 21), has no significance according to this view.

When first differentiated, the nucleolus appears homogeneous with all the stains used; soon, however, this condition changes and a central medullary mass and an enclosing membrane can be distinguished. In the most favorable hæmatoxylin preparations (Plate 6, Fig. 51) the membrane takes a deep stain, the medulla (*med.*) appears granular and more refractive, and outside of the membrane a faintly staining cortex (*ctx.*) can be distinguished. With methyl green and acid fuchsin the medulla is stained red, and is surrounded by a blue rim that is probably composed of both the membrane and the cortex seen in the hæmatoxylin preparations. The next changes consist in simple growth, the medulla, surrounded by its membrane, coming to occupy an excentric position within the cortex, which is increasing more rapidly than the medulla (Plate 6, Figs. 52, 53). Next we have developed within the medulla, and often very close to its membrane, a variable number of very highly refractive bodies (*cp. ref.*). At the same time the rest of the medulla becomes less refractive and more finely granular (Plate 6, Figs. 54, 55). With the methyl green and acid fuchsin combination, the cortex and medulla are with difficulty distinguishable, both taking a pale bluish green stain; but the refractive bodies are colored a very bright pure green. They are therefore chromatin and apparently pure nucleic acid. At this stage,

and for some time later, they are the only structures in the nucleus that take a decided chromatic stain.

After further increase in size, hæmatoxylin stains the cortex deeper and deeper, until it is barely possible to see the medulla and the refractive bodies (Plate 6, Fig. 56). Later this deep staining is still more pronounced, so that both medulla and refractive bodies are entirely obscured and only a faint lighter central area perceived (Plate 6, Fig. 48). During the earlier stages also iron hæmatoxylin usually stains the nucleolus so strongly that no structure can be detected within it; and only exceptionally are the conditions illustrated in Figures 51 to 56 (Plate 6) to be made out. In such instances, however, they may be brought out most distinctly. But with methyl green and acid fuchsin, medulla and cortex are usually differentiated, though not so clearly as with a favorable hæmatoxylin stain.

For the further history of the nucleolus I have depended almost entirely upon the methyl green and acid fuchsin combination. All of the structures increase in volume, the refractive bodies probably most rapidly, until at the time when the yolk bodies are being formed some are considerably over 1 μ in diameter. They still stain a bright green, and are very refractive, but the largest are no longer homogeneous, appearing to be filled with many refractive granules (Plate 6, Fig. 58). Though usually located in the medulla, some are occasionally found within the cortex (Fig. 58). The cortex itself appears perfectly homogeneous, while the medulla is finely granular, but both take the same bluish tint. The medulla is no longer spherical, but flattened on one side.

From now on, while the germinative vesicle is shrinking, and after the yolk is formed, both cortex and medulla take a fainter and fainter stain, until finally they can no longer be distinguished with the use of this stain. During this process they usually are of a diffuse green color, and sometimes the vicinity of the refractive bodies, which remain with unimpaired distinctness, has a faint suffusion of green, which is probably an indication of the presence of what is left of the nucleolus, though its outlines cannot be seen. After the central yolk clump has broken up, this stain has never shown anything of the nucleolus except the refractive bodies; but I think that the other portions are still there, for in the occasional well-stained hæmatoxylin preparations of this stage it is still seen (Plate 6, Fig. 50), though in this case too it is fainter than in the stages immediately preceding.

In addition to the stains already mentioned, I also tried List's

('96, pp. 480–487) potassium ferrocyanide methods in order to determine whether any of the structures in the nucleolus were composed of the paranuclein demonstrated by these methods. List says (p. 488), "Werden die Schnitte eine halbe Stunde lang in eine ganz schwache, mit Salzsäure angesäuerte Eisenchloridlösung gestellt (zu 50 ccm destillirtem Wasser gebe man 10 Tropfen einer 0,5% igen Eisenchloridlösung und 5 resp. 15 Tropfen einer 1% igen Salzsäure), und wird dann die Berlinerblaureaction ausgeführt, so bleiben Nucleïn und die verwandten Stoffe farblos, die Substanz des Nebennucleolus dagegen wird blau. Der name Paranucleïn, gleichsam als Gegensatz zu Nucleïn, ist chemisch daher vollkommen gerechtfertigt." Both his first method, which consists in treating the sections directly with two drops of 1.5% potassium ferrocyanide, followed by one or two drops of hydrochloric acid, and his second method, in which the sections are treated first with the mordant mentioned in the quotation above, were employed, and similar results obtained. The refractive bodies were the only structures that took a decided blue stain, and hence would be called paranuclein, according to List. However, not all the refractive bodies in the same section would be colored blue, but usually only the smaller homogeneous ones. After treatment with potassium ferrocyanide, the sections were stained in Mayer's hydrochloric acid carmine, and the principal result of the cyanide method was to prevent the nucleoli taking the carmine stain. The cortex was acted upon least, and usually took a light, but sometimes a rather deep, stain; but in the nucleoli in which medulla and refractive bodies were differentiated, neither had a red color. They were usually greenish yellow. In the younger ova, with nuclei about 20 μ in diameter, the nucleoli were almost entirely colorless, having but a faint bluish or greenish yellow coloration. The principal effect, then, of List's method is to prevent subsequent coloration of the nucleoli by carmine; but it also stains a few and sometimes all of the refractive bodies blue. These bodies, then, are paranuclein, but we have already seen that, with methyl green and acid fuchsin, they are often the only structure in the nucleus that stains bright green, and hence they are nuclein or nucleic acid. Which is correct? It may be that the refractive bodies represent some chemical substance that has not yet been tested by these reagents, and does not belong in the same category as the nucleins and paranuclein. This, however, is hardly probable, and I think that of the two methods the methyl green and fuchsin is much more reliable on account of the chemical tests to which

it has been subjected; and believe that the refractive bodies are really composed of nuclein or nucleic acid.

Of course the most interesting problem connected with the nucleolus is whether the refractive bodies contain any of the chromatin that goes to form the chromosomes of the maturation nucleus, but on account of the small number of ova that I have found in the oviduct, and the difficulty experienced in making perfect series of sections, I am unable to offer any direct evidence on the subject. One series from an ovum within the oviduct showed three of these bodies situated within the remains of the disintegrated germinative vesicle, but no other distinct structural elements were detected; and when the ovum has reached the pouch, the tetrads (Plate **6**, Figs. 60, 61) are already formed, and no trace of the nucleolus remains. On the whole, it seems rather improbable that the refractive bodies should form the chromosomes, and in the latest ovarian stages there is other chromatic material in the germinative vesicle. Occasionally the whole vesicle will be suffused with a faint green coloration, and sometimes very minute green microsomes are seen on the projections of the shrivelled vesicle. Accordingly, at this stage, there is not the entire absence of other chromatic material that is encountered earlier.

4. Maturation.

The condition obtaining among the ova of Class 4, where the yolk bodies are completely formed and the germinative vesicle much shrivelled, stellate, and about 20 μ in diameter, is the last stage that I have observed in the ovary. The next changes take place in the passage through the oviduct, during which the tetrads are formed.

A few observations have been made on these and subsequent stages, but, owing to the difficulty of obtaining perfect series, they are so scattered that they cannot be easily interpreted, and a discussion of them would be unprofitable. Enough, however, has been seen to show that the number of tetrads in the maturation nucleus is probably twelve, and that two polar bodies are formed. No centrosome or achromatic fibres of any kind have been detected either during maturation or the first cleavage of the ovum, although I have several good preparations of these stages. But, as before mentioned, the number of figures is so small, and the variability in their appearance so great, that as yet no connected history of the processes can be made out.

Cambridge, May, 1898.

POSTSCRIPT.

Since the above was written, Professor Salensky has had the kindness to afford me an opportunity of examining his sections of the embryos of Distaplia magnilarva. As these preparations show a class of facts, not observed in mine, which is of much importance in considering the origin of the cells in the test, their consideration is imperative. But before I proceed to discuss the differences, I will mention two points in which our preparations agree.

1. What I have said about the flattening of the test cells against the ectoderm (p. 86) applies equally well to those of Salensky's preparations that I have seen. The flattening appears to be entirely due to the pressure of the embryonal membranes. Wherever the test cells were flattened and the follicular membrane could be made out at all, the membrane was seen to be pressed against the surface of the embryo; and, conversely, wherever the follicular membrane was seen to lie at some distance from the ectoderm, as where it passes over the tail, the test cells were *never* seen flattened against the ectoderm.

2. The granular substance sometimes found between the test cells in D. occidentalis is also present in D. magnilarva. It seems, in this case also, to be an artifact, being likewise found within the cavities of the embryo, and principally on one side of the embryo. But it is more finely granular and less one-sided in its distribution in D. magnilarva than in D. occidentalis.

The differences between Salensky's preparations and mine are connected with cells situated entirely outside the test-matrix, but having a structure intermediate between that of the cells in the test and the "test cells" (Salensky's kalymmocytes). Whereas, in my preparations, I could find no intermediate stages, in Salensky's quite a perfect transition could be traced from cells that could not be distinguished with certainty from typical kalymmocytes to those looking just like the characteristic vacuolated cells of the test. But among the cells on the outside of the test-matrix the series may be traced even farther, even to the characteristic undifferentiated mesoderm cells with large nuclei and very scanty compact cytoplasm. These facts, and others to be mentioned presently, have forced upon me the conclusion that in *D. magnilarva* the mesoderm cells wander through the ectoderm, not only into the test, but also *on to the surface of the embryo, and there undergo a degenerative vacuolation, the final result of which cannot be dis-*

tinguished from a kalymmocyte. The facts for and against this interpretation may be briefly stated as follows: —

1. The fact that, among the cells entirely outside the embryo, the series may be traced not only to the typical vacuolated cell of the test, but also to the undifferentiated mesoderm cell, tells against Salensky's view that the kalymmocytes become rejuvenated and help to form the test. For, if his view were correct, one would expect that the rejuvenation of the kalymmocytes would cease as soon as they had reached the structure of the typical cell of the test, and that the concentration of the cytoplasm would not go farther, and produce cells not to be distinguished from undifferentiated mesoderm cells. Undifferentiated mesoderm cells are found in the test in comparatively small numbers; and, as it is certain that at least some of them wander through the ectoderm in the undifferentiated condition, and later undergo vacuolation to form the typical cells of the test, it seems probable that sooner or later all the undifferentiated mesoderm cells within the test do the same. If this is true, then the undifferentiated mesoderm cells found on the outside of the test are, according to Salensky's theory, destined to reverse their development once more and become again moderately vacuolated, after having already passed through that stage twice. Of course the considerations adduced in this paragraph do not prove my point, for it may be replied that, as the participation of the kalymmocytes in the building up of the test implies a rejuvenation, it is not at all surprising that some of the cells should become more rejuvenated than is necessary. The above considerations, however, show that the presence of undifferentiated cells on the outside of the test adds a further complication to Salensky's theory.

2. These undifferentiated mesoderm cells are found in such positions, and associated with each other in such a way, that it is difficult to see how they can have been formed from kalymmocytes. Thus, in one case, in an embryo in which none of the cellulose test-matrix had been secreted, an undifferentiated mesoderm cell was found *within* the cytoplasm of a typical kalymmocyte, whose shrivelled nucleus was still distinctly visible. Near by, but still imbedded in the *ectoderm*, was another mesoderm cell of exactly the same appearance, as if to show the course which the first cell had traversed.

In several other places on the surface of the same embryo isolated undifferentiated mesoderm cells were found. The majority, however, of the mesoderm cells thus situated were aggregated into groups. In one case a group of three was encountered, and in another a group of eight

or nine, a part of which was contained in each of two sections. If these undifferentiated mesoderm cells had been developed from kalymmocytes, we should not expect to find them thus massed together, but, instead, distributed at intervals approximately corresponding to the distribution of the kalymmocytes about the embryo. Nor should we expect to find them penetrating other kalymmocytes. It must be admitted, however, that neither of these facts is wholly inconsistent with Salensky's theory.

3. Undifferentiated mesoderm cells were found on the surface of younger embryos, such as had not yet secreted any test-matrix, as well as on the surface of older ones possessing a test of considerable thickness. But while in these older embryos there were transitions to cells that were highly vacuolated, like the kalymmocytes, in the younger ones these transitions were entirely absent. This is exactly what should be expected according to my interpretation, for the cells on the younger embryos have just wandered out through the ectoderm, and have not yet had time to become vacuolated, as they have in the older embryos. I do not see how, according to Salensky's view, these facts can be explained, for if the undifferentiated mesoderm cells have been derived from the kalymmocytes, the transition stages should be present in both cases.

4. The strongest objection to my theory, and one that will immediately present itself to every one, is the great improbability that cells which have had such a different history as typical mesoderm cells and kalymmocytes should both end in structures that cannot be distinguished from each other. It must be remembered, however, that both are of mesodermic origin, and that both are subjected to the same environment. Improbable as such a convergence may seem, it does, however, occur in D. magnilarva; for, in Professor Salensky's sections, I have seen in the body cavity cells, certainly not displaced by the knife in sectioning, which were vacuolated in such a way that only with difficulty could they be distinguished from kalymmocytes, — one, indeed, that had exactly the appearance of a kalymmocyte. The a priori improbability of such a convergence in development is thus shown to be without weight. On the whole, then, I think it must be said that, in spite of the series of transitions from kalymmocytes to cells resembling those in the test (D. magnilarva), this series does not prove that the kalymmocytes participate in the formation of the test.

Berlin, March, 1899.

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EXPLANATION OF PLATES.

All the drawings were outlined with a camera lucida; tube length, 160 mm. projection on the table. Zeiss lenses were used, except where especially specified. *Distaplia occidentalis* is figured in all cases where the name of the species is not mentioned. All the figures were drawn from sections, except where the contrary is stated. The combination of lenses oftenest used was a $\frac{1}{18}$ immersion objective and No. 2 ocular, and these, together with the camera, gave a magnification of 1300 diameters. Where no lenses and magnifications are mentioned this is the combination used.

LIST OF ABBREVIATIONS.

a.	Space due to shrinkage.	*lac.*	Lacuna.
brs. ov'dt.	Oviducal part of incubatory pouch.	*ll.*	Lamella.
brs. pi'brn.	Peribranchial part of incubatory pouch.	*lu. oa.*	Lumen of the ovary proper.
cl. con't. tis.	Connective-tissue cell.	*lu. pd.*	Lumen of the stalk tissue.
cl. fol.	Follicle cell.	*mb. prp.*	Membrana propria.
cl. fol. pr.	Primordial follicle cell.	*med.*	Medulla.
cl. ms'drm.	Mesoderm cell.	*nl.*	Nucleus.
cl. sp. pr.	Primordial sperm cell.	*nl. e'th. fol. i.*	Nucleus of a cell in the inner follicular epithelium.
cl. tst.	Test cell.		
cp. c.	Central corpuscle.	*nll.*	Nucleolus.
cp. ia'll.	Intralamellar body.	*oa.*	Ovary.
cp. ia'vac.	Intravacuolar body.	*oa-te.*	Ovotestis.
cp. ia'vt.	Intravitelline body.	*ov.*	Ovum.
cp. lut.	Corpus luteum.	*ov'dt.*	Oviduct.
cp. ref.	Refractive body.	*ov'go.*	Oögonium.
cp. vt.	Yolk body.	*pd.*	Stalk or peduncle.
ctx.	Cortex.	*par'.*	Superficial wall of the ovary.
cyt'pl.	Cytoplasm.		
ec'drm.	Ectoderm.	*par''.*	Deep wall of the ovary.
en'drm.	Endoderm.	*sac. brn.*	Branchial sac.
e'th. fol.	Follicular epithelium.	*sac. pi'brn. d.*	Right peribranchial sac.
e'th fol. ex.	Outer follicular epithelium.	*sac. pi'brn. s.*	Left peribranchial sac.
		stg.	Stigmata.
e'th. fol. i.	Inner follicular epithelium.	*te.*	Testis.
		tis. pd.	Stalk tissue.
e'th. g.	Germinative epithelium.	*tst.*	Test.
fun. con't. tis.	Connective tissue strand.	*vac.*	Vacuole.
fun. cyt'pl.	Cytoplasmic strand.	*va. df.*	Vas deferens.
fun. gen.	Genital strand.	*vs.*	Vesicle.
gra. chr.	Chromatin granules.	*vs. ex.*	Outer vesicle.
in.	Intestine.	*vs. i.*	Inner vesicle.

PLATE 1.

Fig. 1. Undifferentiated bud.

Fig. 2. Ovotestis of a young bud, showing ovary and testis separated, and the beginnings of a peripheral epithelium about the latter.

Fig. 3. Section immediately anterior to Fig. 2. Ovary and testis are united.

Fig. 4. Sagittal section of a medium sized bud. Ovary and testis differentiated, and both attached to the genital strand.

Fig. 5. Cross-section near the posterior end of the abdomen of an adult zoöid, showing the ovary with its lumen proper, and the lumen of the stalk tissue, and a corpus luteum. D, 3; × 530.

Fig. 6. Same, from another zoöid, showing the ovary. D, 3; × 530.

Fig. 7. Part of the germinative epithelium of an adult zoöid, showing a young oögonium projecting into the lumen of the ovary.

2
par
ov.go
lu oa
par
lu spd
lu pd
mb prp
7
par
ep ta'rt
ep tut
lu oa
ec drm
par
lu oa
lu pd
par
ns pd
ov.go
ov.go
6
4
gra.chr
ov.go
oa
el sp pr
el fol pr

PLATE 2.

Fig. 8. Whole preparation of a young incubatory pouch, about $\frac{1}{4}$ adult size. A, 2; × 91.

Figs. 9, 10. Cross-sections of the posterior end of the thorax of a zoöid that had nearly reached maturity, showing the region where the stalk of the incubatory pouch joins the zoöid. Fig. 10 is the more anterior section. D, 2; × 380.

Fig. 11. From another zoöid of the same age as that figured in Figs. 9, 10, showing the opening of the pouch into the peribranchial sac. D, 2; × 380.

Fig. 12. Cross-section of the ovary and testis of a young *Styela montereyensis*. Powell and Leland $\frac{1}{12}$ immers.; × 850.

9.
va.df.
ov'dt.
a.
a.
brs. ov'dt
brs. pi'brn.
ec'drm.
tst
10.
va.df
ov'dt
ec'drm
brs. pi'brn.
8.
brs.pi'brn.
ec drm
brs ov'dt.
stg.
sac.brn.
sac.pi'brn d.
11.
brs.pi'brn
va df
in
ec'drm
par
te
12
eth.sac.pi'brn

PLATE 3.

Fig. 13. Oögonium from an adult zoöid. The test cells are just forming.
Fig. 14. Part of the wall of the ovary, showing an ovum projecting beyond the ovary and just beginning to form a stalk.
Fig. 15. Same; ovum is much older, with a well-formed stalk containing differentiated stalk tissue.
Fig. 16. Whole preparation of ovum, showing maximum size attained in the ovary. Bausch & Lomb 2 in., Zeiss 2; × 40.
Fig. 17. Same; ovum partly extruded from ovary into oviduct. Bausch & Lomb 2 in., Zeiss 2; × 40.
Fig. 18. Same; ovum has not quite completely entered the pouch. Bausch & Lomb 2 in., Zeiss 2; × 40.
Fig. 19. Section through a medium-sized ovum, showing bends in the nuclear membrane and cytoplasmic vacuoles.
Fig. 20. Germinative vesicle of an ovum in a medium-sized bud.
Fig. 21. Ovum in a medium-sized bud.
Fig. 22. Young ovum from an old bud.
Fig. 23. Same; ovum a little older.
Fig. 24. Follicle and periphery of an ovum a little larger than that in Fig. 13. Test cells are being formed.
Fig. 25. Test cell and follicle cell from the same ovum.
Fig. 26. Follicle and test cells of a medium-sized ovum. A much smaller ovum is enclosed within the thickness of the follicle.

17.
el.tst
16.
el.tst
17
el
hs.vdlt
18.
20.
vac
19.
mb.tul.
21
22.
vac
23.
epia'vt
14.
25.
24.
el.tst.
ovijo
hs.st
hs.pd
15.
ot
26.
hs.ext.pl
el.tst
hs.pd
pd
hs.pd
hs.pd
eth.tul

PLATE 4.

Fig. 27. Ovum in which the test cells and secondary follicular epithelium have just become differentiated. Reichert $\frac{1}{12}$ immers., Zeiss 3; × 1240.

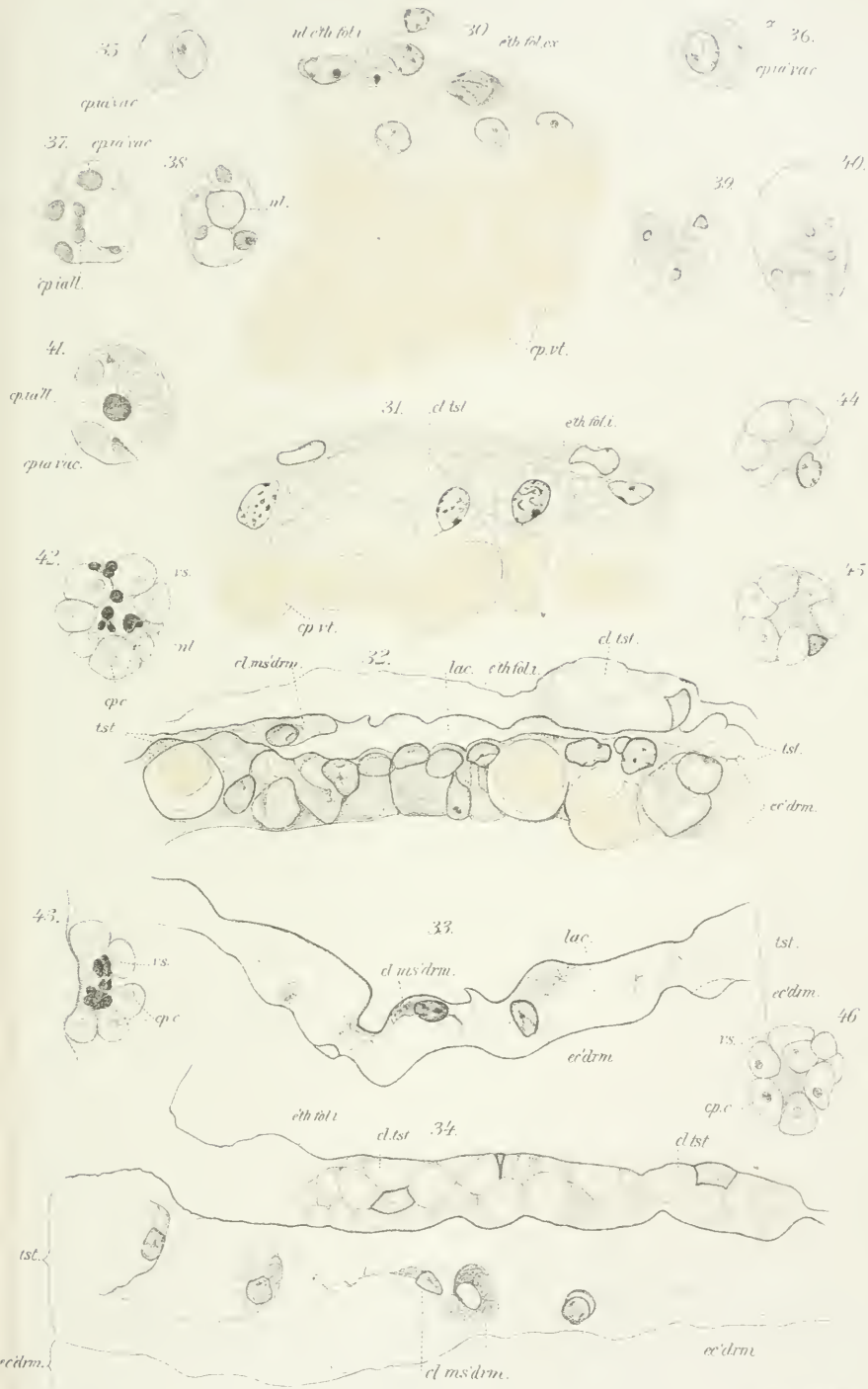
Fig. 28. Part of a half grown ovum (173 × 150 μ), showing the formation of the yolk, follicle, and test cells.

Fig. 29. Selected test cells from the same ovum.

27
cl.tst
e'th.fol.
29.
cp.vt.
cp.a.vt
28

PLATE 5.

Fig. 30. Periphery of an old ovum and its follicle.
Fig. 31. Periphery and follicular envelope of an ovum that has just reached the pouch.
Fig. 32. Ectoderm and test at an early stage in the formation of the test.
Fig. 33. Section of the test when a little more developed.
Fig. 34. Test, still farther developed.
Figs. 35–46. Various stages in the degeneration of the test cells of *Styela montereyensis*.
Fig. 35. Ovum containing the test cell is $84 \times 65\,\mu$. Test cell shows vacuoles and intravacuolar bodies.
Fig. 36. Ovum $92 \times 69\,\mu$.
Fig. 37. Ovum $108 \times 69\,\mu$.
Fig. 38. Test cell from the same ovum.
Figs. 39, 40. Test cells, from another ovum, showing the formation of the central corpuscle within the intravacuolar bodies.
Fig. 41. Ovum $96 \times 80\,\mu$.
Figs. 42, 43. From an ovum measuring $134 \times 111\,\mu$.
Fig. 44. Ovum $92 \times 76\,\mu$.
Fig. 45. Ovum $169 \times 80\,\mu$.
Fig. 46. Ovum, nearly mature, measuring $108 \times 92\,\mu$.


35.
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36.
epia'vac
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epia'vac
epiall.
38.
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epiall.
epia'vac.
31.
cl tst
eth fol i.
44.
42.
vs.
ml
cp vl.
cl ms'drm.
32.
lac.
eth fol i.
cl tst.
cpc
tst
tst.
ec'drm.
45.
43.
vs.
cp c
33.
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cl ms'drm.
tst.
ec'drm.
46.
vs.
cp c
ec'drm.
eth fol i
cl tst
34.
cl tst
tst.
ec'drm.
ec'drm
cl ms'drm.

PLATE 6.

Fig. 47. Part of the wall of a corpus luteum from which the ovum has just passed out.

Fig. 48. Germinative vesicle just as it is beginning to shrink. From the same ovum as that in Fig. 28, measuring 173 × 150 μ. Reichert $\frac{1}{12}$ immers., Zeiss 2; × 890.

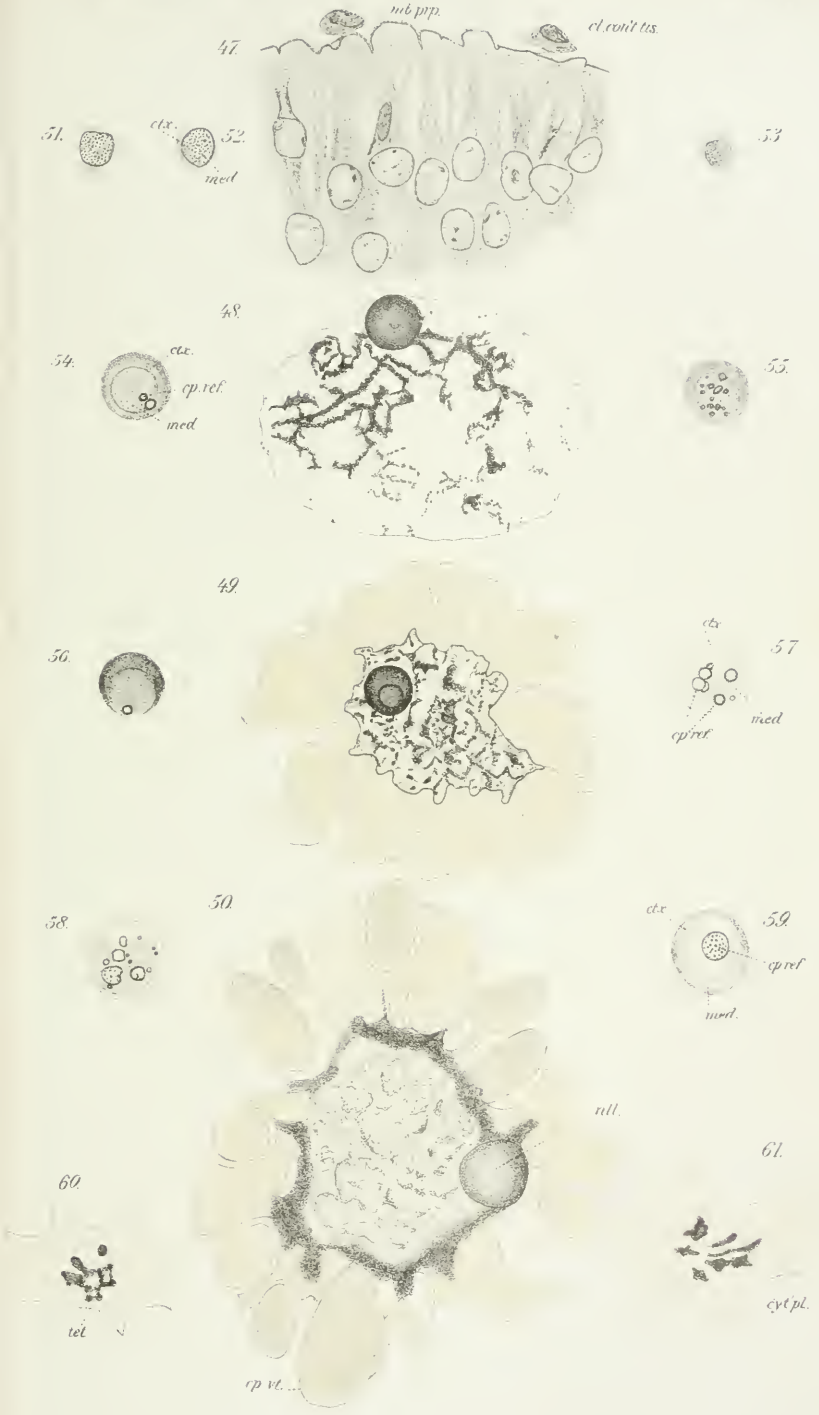
Fig. 49. Germinative vesicle and yolk-clump in an ovum of 190 μ diameter. Reichert $\frac{1}{12}$ immers., Zeiss 2; × 890.

Fig. 50. Germinative vesicle and central yolk bodies at a later stage. Ovum is 254 μ in diameter. Zeiss $\frac{1}{18}$ immers., 2; × 1300.

Figs. 51–59. Nucleoli in various stages of development. The measurements are in μ.

	Dimensions of the Ovum.	Dimensions of the Germinative Vesicle.
Fig. 51.	indistinct	15 × 11
Fig. 52.	23 × 13	13 × 11
Fig. 53.	indistinct	13 × 10
Fig. 54.	46 × 38	25 × 19
Fig. 55.	43 × 34	22 × 18
Fig. 56.	45 × 35	23 × 17
Fig. 57.	191 × ?	33 × 25
Fig. 58.	152 × 113	49 × 30
Fig. 59.	207 × 190	32 × 22

Figs. 60, 61. Two successive sections through the group of tetrads formed just before the formation of the first polar cell. The ovum has just reached the pouch.


47.
mb.ppp.
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51.
ctx.
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57
cp.ref.
med
50.
58.
59.
ctx.
cp.ref
med.
tell.
60
61.
tel
cyt.pl.
cp.vt.

THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[3] XV.[16] The Stalk-eyed Crustacea.

S. GARMAN. The Fishes.

W. GIESBRECHT. XVI.[15] The Copepods.

A. GOËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

H. LUDWIG. IV.[5] XII.[14] The Holothurians.

C. F. LÜTKEN and TH. MORTENSEN. The Ophiuridæ.

OTTO MAAS. XXI.[21] The Acalephs.

J. P. McMURRICH. The Actinarians.

E. L. MARK. XXIV.[24] The Cerianthidæ.

GEO. P. MERRILL. V.[6] The Rocks of the Galapagos.

G. W. MÜLLER. XIX.[19] The Ostracods.

JOHN MURRAY. The Bottom Specimens.

A. ORTMANN. XIV.[12] The Pelagic Schizopods.

ROBERT RIDGWAY. The Alcoholic Birds.

P. SCHIEMENZ. The Pteropods and Heteropods.

W. SCHIMKÉWITSCH. VIII.[8] The Pycnogonidæ.

S. H. SCUDDER. VII.[7] The Orthoptera of the Galapagos.

W. PERCY SLADEN. The Starfishes.

L. STEJNEGER. The Reptiles.

TH. STUDER. X.[10] The Alcyonarians.

C. H. TOWNSEND. XVII.[17] The Birds of Cocos Island.

M. P. A. TRAÜTSTEDT. The Salpidæ and Doliolidæ.

E. P. VAN DUZEE. The Halobatidæ.

H. B. WARD. The Sipunculoids.

H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June 1891. 16 pp. ; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates.

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull. M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z., Vol. XXV., No. 1. September, 1893, 25 pp.

[8] Bull. M. C. Z., Vol. XXV., No. 2. December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4. January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C. Z., Vol. XXV. No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV. No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z. Vol. XXV. No. 10, October, 1894, 109 pp., 12 Plates.

[14] Mem. M. C. Z., Vol. XVII. No. 3, October, 1894, 183 pp. 19 Plates.

[15] Bull. M. C. Z., Vol. XXV. No. 12. April, 1895. 20 pp., 4 Plates.

[16] Mem. M. C. Z. Vol. XVIII., April, 1895. 292 pp., 67 Plates, 1 Chart.

[17] Bull. M. C. Z., Vol. XXVII., No. 3, July, 1895. 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol. XXVII., No. 4, August, 1895. 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895. 14 pp., 3 Plates.

[20] Bull. M. C. Z., Vol. XXIX., No. 1, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M. C. Z., Vol. XXIII., No. 1, September, 1897. 92 pp., 15 Plates.

[22] Bull. M. C. Z. Vol. XXXI., No. 5, December, 1897. 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol. XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M. C. Z., Vol. XXX I., No. 8, August, 1898, 8 pp., 3 Plates.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, July 1899, 4 pp., 1 Plate.

PUBLICATIONS

OF THE

MUSEUM OF COMPARATIVE ZOÖLOGY

AT HARVARD COLLEGE.

There have been published of the Bulletins Vols. I. to XXXI., and XXXIII.; of the Memoirs, Vols. I. to XXII.

Vols. XXXIV. and XXXV. of the Bulletin, and Vols. XXIII. and XXIV. of the Memoirs, are now in course of publication.

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The following publications are in preparation: —

Reports on the Results of Dredging Operations from 1877 to 1880, in charge of Alexander Agassiz, by the U. S. Coast Survey Steamer "Blake," Lieut. Commander C. D. Sigsbee, U. S. N., and Commander J. R. Bartlett, U. S. N., Commanding.

Reports on the Results of the Expedition of 1891 of the U. S. Fish Commission Steamer "Albatross," Lieut. Commander Z. L. Tanner, U. S. N., Commanding, in charge of Alexander Agassiz.

Contributions from the Zoölogical Laboratory, in charge of Professor E. L. Mark.

Contributions from the Geological Laboratory, in charge of Professor N. S. Shaler.

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For the Memoirs, $8.00 " " "

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OCT 18 1899

Bulletin of the Museum of Comparative Zoölogy
AT HARVARD COLLEGE.
Vol. XXXV. No. 5.

OBSERVATIONS ON NON-SEXUAL REPRODUCTION IN DERO VAGA.

By T. W. Galloway.

With Five Plates.

CAMBRIDGE, MASS., U.S.A.:
PRINTED FOR THE MUSEUM.
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No. 5. — *Observations on Non-sexual Reproduction in Dero vaga.*[1]

By T. W. Galloway.

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1. Introduction.

The worm upon which the present study is made was originally described by Leidy ('80) as Aulophorus vagus. A more complete account of the anatomy and histology by Reighard ('84) makes it apparent that its divergence from the species of Dero is not sufficient to warrant the establishment of a new genus. The sexual features which I have recently had the fortune to observe confirm this view.

The excellent work of von Bock ('97) on budding in Chætogaster diaphanus, in which there is a review of the work of preceding authors upon non-sexual reproduction in worms, renders it unnecessary for me to summarize previous accounts here. His interpretations are, however, in some respects so diverse from those of earlier authors as to make it desirable that they should be tested in another group. Dero is one of the most specialized of the Naidiform Oligochæta, and for this reason presents certain interesting variations from the conditions encountered in the forms that have been previously studied.

In the autumn, from September to November, Dero vaga is found abundantly in tubes formed of Lemna leaves, or other small light objects, at the surface of water in the ponds and ditches in the environs of

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. XCIX.

Cambridge. At this time it usually occurs as single individuals or with only two zoöids in a chain. These, collected and placed in a suitable aquarium, will thrive and multiply asexually throughout the winter. The rate of division depends upon the food supply, by the regulation of which certain interesting variants from the normal may be had.

2. Structure of the non-dividing Animal and the Formation of Segments.

In individuals not sexually active and not in process of budding, the following regions may be distinguished: (1) prostomium; (2) four cephalic segments bearing ventral bristles only; (3) twenty to twenty-five well developed body segments, with both dorsal and ventral bristle bundles; (4) a region of incompletely formed segments passing posteriorly into an undifferentiated region or zone, — all of which may equal two or three adult body segments in length; and (5) a completely developed, complex structure, — representing probably at least one segment (anal segment), — which bears the anal orifice, the respiratory lobes (pavilion), and the digitiform appendages.

The formation of new segments in such a worm is a process which presents so many points of similarity to that of budding, that I wish to give a brief description of it before proceeding to the consideration of the latter phenomenon. New segments are invariably formed immediately in front of the anal segment, and always from the anterior portion of the undifferentiated zone. The length of this preanal zone is greater in well nourished than in poorly nourished individuals. In this part of the body all the structures which characterize segments in the more mature regions are less and less differentiated as one proceeds posteriorly. Even the organs which pass through this region in a functionally complete condition, such as the intestine, the blood-vessels, and, in part, the nerve cord, present simpler conditions than they do farther forward. The nerve chain, for example, is represented chiefly by non-fibrous elements, only a few fibres passing through to innervate the pavilion. The ventral and lateral portions of the body cavity are shown, by transverse sections, to be filled with a mass of indifferent tissue derived from cell multiplications in the ectoderm, the products of which break through the muscular layers at definite places and then fuse together, much as in the budding process (Plate **2**, Figs. 8, 9, Plate **3**, Fig. 15) described later. These ectodermal ingrowths are arranged serially in the long axis of the worm, and afford the earliest signs of segmentation.

3. Position of the Bud-zone: Primary and Secondary.

In such a worm, if the food supply be normal, a budding zone is formed some distance anterior to the preanal or segment-forming zone. Bourne ('91) has suggested that the segment at which bud formation takes place is probably constant for each species. This is certainly not the case here, at least not for the asexually produced zoöids. I am not yet able to say what may be true of the sexually derived individuals, for I have not as yet discovered how, if at all, the sexually produced zoöid may be certainly distinguished from the non-sexually produced forms. That probably can be settled satisfactorily only by rearing young worms from fertilized eggs.

The cellular activity accompanying budding, it is to be observed, involves only one segment, and the plane of separation is always midway between dissepiments. The position of this budding segment in the individuals which I have examined is variable within certain narrow limits. It may occur as far forward as the 16th and as far backward as the 21st setigerous segment. The following table, representing observations upon about three hundred individuals, gives in percentages the frequency of occurrence in these two and the intervening segments.

TABLE I.

Setigerous Segment	16th	17th	18th	19th	20th	21st
Percentage of Occurrence	7.6	15 3	38.2	26.7	7.6	4.6

Table II. is a record of observations upon the position of the budding segment in successive generations.

TABLE II.

Primary Division occurs in		Secondary Division occurs			
		Anterior Zoöid, in		Posterior Zoöid, in	
Segment No.	No. of Cases.	Segment No.	No. of Cases.	Segment No.	No. of Cases.
20	1	20	1	20	1
19	4	19	4	19	2
				18	1
				17	1
18	4	18	4	19	1
				18	1
				17	1
				16	1
16	1	16	1	17	1

It will be observed from this table that, when the anterior zoöid again buds, the zone of budding occurs at the same distance from the anterior end as in the first instance, i. e. in the same body segment. This is not true, however, for the posterior zoöid. There may be either an increase

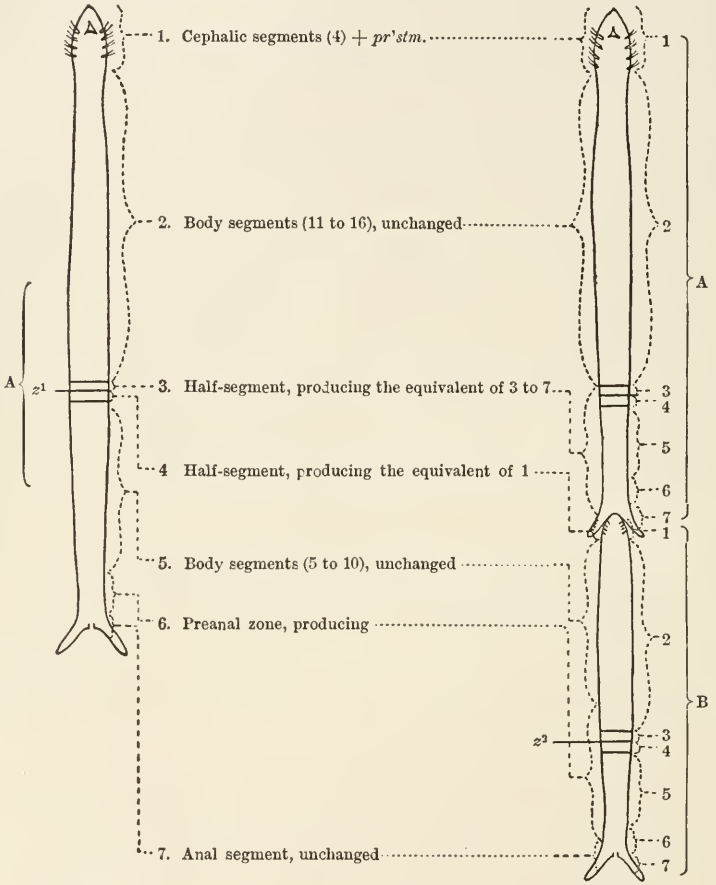

Fig. I. Fig. II.

or a decrease in the number of segments in front of the budding zone as compared with the condition in the parent. This variability is perhaps correlated with the rate of growth as dependent upon food, etc.

It will be seen from the accompanying diagram (Figures I. and II.) that the half-segment *A*, 3 (Fig. I.) produces *A*, 3–7 (Fig. II.), which

simulates the condition of *A*, 3–7 (Fig. I.), and consequently that the second new individual, which is thus to be produced at the posterior end of *A* (Fig. I.), arises from a region which was undifferentiated during the first budding. The half-segment 4 (Fig. I.) produces a prostomium and four cephalic segments, *B*, 1 (Fig. II.). At the same time, the indifferent preanal zone, 6 (Fig. I.) is adding segments to the region 5, which before these additions embraces a small but variable (5–10) number of body segments. Since the budding in no case takes place in a segment so near the head as the tenth body segment, it follows that this must occur in the undifferentiated region 6 (Fig. I.), and consequently it is true for the posterior as well as the anterior zoöid that, when a new budding takes place, it occurs in a zone that was undifferentiated during the preceding process of budding.

4. Relation of Budding to Formation of Segments.

If the worms are well nourished, the secondary divisions often commence before the first is completed. A condition of this sort is shown in Plate **1**, Fig. 3. In such instances the secondary bud (z^2) in each zoöid lies within the limits, either of the indifferent zone (anterior zoöid), or of still incompletely differentiated segments (a condition more usually found in the posterior zoöid).

There is thus shown to be a close relation between the process of budding and that of forming new segments. For the material which is destined to subserve the process of budding is at one time an indistinguishable part of the zone (preanal) the posterior part of which is a continuous source of new segments. In every case the budding zone, on its part, gives rise to two segment forming regions: a posterior, which is normally limited to the production of four segments beside the cephalic lobe; and an anterior, which never loses the power of forming new segments, since some of the segments thus produced, or at least one of them, retain the capacity of forming a new bud, by which the process is repeated and the power perpetuated indefinitely. In other words, the relation of the two processes in this worm is such as to lend support to the view that here budding is a specialized form of normal segmentation. The cycle of procedure occurs just as if, in the formation of segments from the indifferent preanal zone, there were deposited at a certain stage, in one or several segments, materials which are capable under appropriate circumstances of giving rise to indifferent tissue and to new segments.

This fact allies itself with what is known of regeneration of lost parts.

As several authors have shown, the likelihood of the regeneration of a given portion is decreased proportionally to the distance of the regenerating region from the part whose reproduction is expected. For example, the head segments in the earthworm are much more likely to be regenerated from anterior segments than from middle or posterior ones. In this case, too, the behavior suggests, one might say, a diminution of head-producing material posteriorly.

In addition to this normally recurrent relation between these two regenerative processes in Dero, I have found in two cases that, if a large number of posterior segments are removed from a worm in which the budding zone has just begun, the process of budding continues, without any effort being made to regenerate the lost tail piece. In such cases, however, the budding departs from the normal course, since the proliferations in what should become the head of the posterior zoöid are amorphous, none of the normal structures being produced; but the anterior half of the budding segment produces an indifferent zone and an anal segment in the customary way, with, however, minor imperfections. Here the behavior is as if the regenerative process had been transferred, as a matter of economy, to the budding region instead of taking place where the injury existed. In a case where only a small number of segments were removed, the cut being immediately in front of the indifferent preanal zone, regeneration occurred at the point of cutting, and the ectodermal thickening of the budding zone disappeared. After the regeneration of the tail piece was completed, the animal formed again the budding zone in the original segment (in this instance the 18th setigerous) and ultimately divided.

It is a suggestive fact, that, furthermore, in the sexual individuals, *some* of which are known to be asexually produced, I have found that the testes are formed upon the posterior face of dissepiment iii/iv, i. e. on a new dissepiment produced in the process of budding, in a region which was of course located in the parent individual much posterior to the usual place of production of gonads. The ovaries arise likewise upon the posterior wall of the dissepiment between setigerous segments iv/v. Although this is one of the original dissepiments, the new tissues are closely related to it in their origin and development.

5. Experiments on the Rate of Budding.

In the effort to secure an abundance of budding material, there appeared certain facts of practical and perhaps theoretical importance

relative to the rate of division. Two classes of culture solutions were used in rearing the worms. Algæ and other organic substances native to pond water were supplied in the first; the second consisted of an infusion of boiled corn-meal in water in the proportion of 1 : 1000. Of the first, three grades were roughly distinguished: (1) that in which the organic matter was in such excess that striking evidence of decomposition was sure to occur; (2) that in which the organic matter was present in such quantities that no conspicuous impurity arose from its decomposition; and (3) that in which the organic material was reduced to a small per cent of what could have been employed without signs of decomposition. In the first solution, bacteria, paramœcia, and stentors appeared in great abundance, and it became necessary from time to time to pour a part of this away and renew the water. In the other two cases the water was not disturbed except as evaporation made renewal necessary. In the corn-meal solution frequent changing was necessary on account of rapid fermentation.

The following tabulated statement represents the results, but gives of course only rough quantitative data as to the effect of the food supply in

10 Individuals in each Culture.	Pond-water, with Algæ and other organic Food Supply.						Corn-meal in Water.	
	A. In Excess.		B. Medium.		C. Minimum.		D. 1 : 1000 parts.	
	15 days.	30 days.	15 days.	30 days.	15 days.	30 days.	10 days.	20 days.
Exper. 1	18	23	15	28	13	15	18	25
Exper. 2	22	19	18	30	12	14	20	22
Exper. 3	21	22	14	26	15	16		
Average	20.3	21 3	15.6	28	13 3	15	19	23.5
	1st 15 days.	2d 15 days.	1st 15 days.	2d 15 days.	1st 15 days.	2d 15 days.	1st 10 days.	2d 10 days.
Percentage Increase	103%	5%	56%	80%	33%	13%	90%	23 5%

influencing growth, as measured by the rate of germination. In each experiment, at the outset, ten individuals were taken approximately at the same (an early) stage of division. The figures in the columns of "days" indicate the number of separate worms at the end of the time

indicated. The "30 days" columns take no cognizance of the stages of division of the individuals occurring at the end of the "15 days" periods, and for this reason the two columns of each culture are perhaps not strictly comparable. The horizontal line of percentages is reckoned upon the number of individuals existing at the beginning of the period in question.

It will be seen that during the first fifteen days solution "A" was productive of the most rapid division, in some instances the second division being accomplished; but during the second period the worms scarcely more than held their own. This is seemingly due to the prevalence of bacteria. Culture "D" presents somewhat similar phenomena. In "C" the falling off in the second period is probably caused by the disappearance of food from the water and from the tissues of the body. "B" is the control culture, and more nearly represents the ordinary rate of reproduction under favorable conditions. These are winter cultures. Propagation would doubtless be more rapid with fresh worms in summer or fall.

6. Methods.

Dero does not present any special technical difficulties except that, in the later stages of budding, separation of the zoöids is likely to occur in the process of killing, whereby control of the material is lost. I used as killing and fixing reagents with about equal success (1) a saturated aqueous solution of corrosive sublimate plus 1% acetic acid (hot), and (2) a solution of hot picro-sulphuric acid (Kleinenberg's). Staining of excellent quality was secured by Heidenhain's iron-hæmatoxylin method. Sections were made from 6 to 12 μ in thickness.

7. Histological Features of the Budding Process.

In the discussion of the rôle played by the different embryonic layers in the production of the new organs made necessary by budding, I shall first treat in a general way of the changes occurring in each layer, and the organs which are wholly developed therefrom. Afterward, at the risk of some repetition, I shall correlate the share of each layer in the formation of the mouth, and such other structures as involve more than one germinal layer.

a. Ectoderm.

The first evidence of the bud-zone, as seen in optical sections of the living worm, is a slight thickening of the ectodermal elements of the dermo-

muscular wall (Plate **1**, Fig. 2, *z'*; Plate **2**, Figs. 11, 12). The thickening is first manifest on the ventral aspect, extending ultimately as a girdle around the segment. It is interseptal in position, and about equally distant from the nearest dissepiments. That this thickening is a matter of growth and not of local contraction is shown both by the increased number of cells and the increased length of the segment. The peripheral margins of the dissepiments bounding the bud-zone are crowded apart, as indicated in Figure 2. The bristle bundles belonging to the involved segment are forced backward into contact with the following dissepiment. They become the bundles of the 5th setigerous segment of the posterior zoöid. An external groove (Plate **1**, Figs. 4, 5, *sul.*) is next formed in the ectodermal thickening, and persists as the outward demarcation between the zoöids, indicating the place of ultimate separation. This groove deepens gradually but unequally at different points of its circumference. The activity of the tissue is strictly confined to one segment, the segments in front of and behind this one undergoing no appreciable changes. Hence, it is possible to affirm that the tissues of the active segment posterior to the plane of the groove furnish the material out of which are formed the prostomium, four new segments, and the anterior portion of the fifth segment, with their contained structures. Similarly, from the anterior half of the segment spring the whole set of structures appropriate to the tail end of the worm. It is possible to locate the fundaments of these organs from the beginning of the process.

The division of ectodermal cells may continue until a thickness of three or four cells is reached in the dermal layer. In the mean time, as may be seen in transverse sections, the proliferating ectodermal tissue breaks through the muscular layer into the body cavity, especially in the spaces between the longitudinal muscle bands (Figs. 8–10). These invading elements on either side the median plane coalesce with each other and with proliferations of the cells from the ventral nerve cord, filling the ventral and lateral portions of the body cavity in a very characteristic way (Plate **1**, Figs. 6, 7). In a sagittal section, a differentiation of this internal cell-mass into an anterior and posterior portion is apparent at a relatively early stage (Fig. 5). The cells on the contiguous faces of these masses become arranged into bounding surfaces, which are ultimately continuous with the external layer, and become, in part, the boundaries of the deepening groove. Thus the latter is supplemented in its deeper portion by an internal delamination, and the two together produce deep invaginations, especially from the latero-ventral regions (Plate **3**, Figs. 16, 17, *sul.*), which are paired and contribute to

the formation of the mouth. The apparent depth of the ectodermic depression is increased by a pair of growths (directed backward and outward) of the thickened ectoderm anterior to the groove in the latero-ventral regions, to form the digitiform appendages (Figs. 16, 17, *pr'c. dg.*).

The process of ectodermic ingrowths between and through the muscle bands, referred to above, is kept up both anterior and posterior to the plane of separation of the zoöids.

In the *posterior* zoöid there are five distinct regions of cellular activity in the ventral part of the body, namely: (1) the ectoderm in the spaces between the lateral and ventral bands of longitudinal muscles (Plate **1**, Figs. 6, 7; Plate **2**, Figs. 8, 9); (2) the ectoderm invading the ventral muscle band and cutting off certain fibres (Plate **1**, Figs. 6, 7, *mu. v.* and *mu. v'.*) at either margin; and (3) the cellular elements of the ventral nerve cord itself. More dorsally, in the spaces between the dorsal and lateral longitudinal muscles, there is likewise an ingrowth of ectoderm on either side of the body, which in some measure supplements and fuses with the masses already described. More dorsal still, approximately at a level with the dorsal wall of the gut, is seen (Fig. 7) another irruption of ectoderm so located as to cut off a strand from the ventral margin of the dorsal muscle. This ingrowth differs from the former ones in the very important fact that its extent in an antero-posterior direction is extremely limited. The former proliferations occur one after another in longitudinal series, one of each series to every segment. The ingrowth through the dorsal muscle band, on the contrary, is limited to a single pair of cell masses situated well forward toward the plane of division. The cells from this pair of ingrowths move dorsally and toward the median plane until they rest upon the digestive tract, and finally meet each other, forming the brain ganglia, whose elements later produce a connective (Plate **1**, Fig. 6, *con't. crc'œ.*). The detached portion of the dorsal muscle band approximately equals in dimensions the whole lateral band. The pair of irruptions through the marginal portions of the ventral muscle, in conjunction with elements from the ventral nerve cord itself, furnishes the material for the cord in the newly forming segments of the anterior zoöid and the infra-pharyngeal enlargements of the nerve cord in the posterior zoöid. In the posterior worm there is a degeneration of that part of the old nerve tract which lies in front of the place to be occupied by the infra-pharyngeal ganglia, only a few fibres remaining to preserve connection between the individuals until separation is accomplished.

The ingrowths between the ventral and lateral muscle bands give rise, in each of the four cephalic segments of the posterior individual, to a pair of ventral bristle sacs, and to the ectodermal part of the nephridial organs. There are no dorsal bristle bundles formed in the cephalic segments of the posterior zoöid.

The fate of the proliferation between the dorsal and lateral longitudinal muscle bands is more obscure. The circumœsophageal connective, in running from the brain ventrally, passes, as has been said, between the main portion of the dorsal muscle band and the lateral part of it, which is thereby split off from the main band. In its further course ventrad, the connective again returns to a position inside the longitudinal muscles. This it does by running inward between the split off portion of the dorsal band and the lateral band of muscles, so that no part of the lateral muscle band ever lies inside the connective. It is perhaps significant that the cells of the lateral line make their appearance in the same space between the dorsal and lateral muscle bands (Plate **1**, Figs. 6, 7; Plate **2**, Fig. 10, *prf.* and *cl. ln. l.*). The two sets of nervous structures, — the circumœsophageal connective and the lateral line cells, — if the latter are really nervous, are thus apparently in relationship by their proximity. It is, furthermore, possible that the cells which push in and go to form the brain do so here rather than at the more dorsal position previously noted. The fact that the whole ectodermal arc peripheral to the detached portion of the dorsal band is very much thickened would seem to support this view. In this event, we should be compelled to admit that the future nervous elements produced at this place grow dorsally in a position peripheral to the cut off portion of the dorsal longitudinal muscle band, between which and the rest of the dorsal band they pass into the body cavity. This would help to explain the course taken by the fibres of the connective in uniting the brain and subpharyngeal ganglion. It would also connect the brain in its origin with the lateral line system, rather than with the ventral chain, the connection with the latter being an altogether secondary one. However this may be, the course of the nervous connective is associated with the position of the lateral line cells in a very suggestive way. I am as yet unable to state what, histologically, the origin of the connective fibres may be. From analogy with other annelids we should expect them to arise from the cells of the brain, or possibly from the subœsophageal ganglia, or both. The course of the connective is shown semi-diagrammatically in Figure 10, *con't. crc'œ.*

In the *anterior* zoöid the course of development is somewhat different.

The ectodermic invasions occur in the same way as in the posterior zoöid and in corresponding positions, but the one which cuts off a lateral portion of the dorsal muscle band is ultimately repeated here for each new segment, and occurs at the place where subsequently the dorsal bristle bundles are found. In fact, these ingrowing elements constitute the bristle forming organs. It is further to be noted that, at the time of separation of the zoöids, the bristle bundles of the anterior worm are in a much less advanced state of development than those of the posterior worm. Another point of difference is found in the fact that there is a much more intimate fusion of the several ectodermic ingrowths of the ventral and lateral regions with each other and with mesodermic elements in the anterior than in the posterior zoöid. In this manner is produced an indifferent zone, similar in all particulars to the undifferentiated segment-forming zone previously mentioned as characteristic of the preanal region. From the ectoderm comes, as in the posterior zoöid, the mass of tissue from which ventral nerve chain, ventral bristle sacs, etc., are developed. All stages in the differentiation of these structures may be found in a rapidly growing worm, as one passes forward. Immediately in front of the anal segment, where the tissue is least differentiated and the ventral nerve cord is to be developed almost *de novo*, an ectodermic ingrowth in a mid-ventral position divides the ventral longitudinal muscle and contributes to the ventral cells of the cord (Plate **3**, Fig. 15; Plate **4**, Fig. 23). Proliferations which are lateral, but still penetrate the ventral muscle, add cellular elements to the margins of the cord (Plate **2**, Fig. 8; Plate **5**, Fig. 24, *gn. v.*). Thus in the formative region the cord can be resolved into a median and two lateral constituents. It was the latter which Semper ('76) regarded as mesodermal. The fibres of the nerve cord naturally become less numerous posteriorly. The more dorsal, i. e. the deeper fibres, are the first to appear; this produces the condition figured in Plate **3**, Fig. 15, in which the fibrous tract appears to occupy a more and more dorsal position as one proceeds toward the tail. The ectoderm on the ventral side of the body in this region is especially thickened, being four or five layers of cells deep (Figs. 15, 16, 22, 23, 24).

b. Entoderm.

It is to be borne in mind that before the beginning of division the digestive tract in the region affected by that process is a simple tube with interseptal enlargements. The wall of the intestine, reckoning from the

lumen outward, here consists of (1) a layer of ciliated epithelium, in which the free ends of the cells form a wavy contour; (2) scattered sub-epithelial cells lying in the basal portion of this epithelium (Plate **2**, Fig. 13); and (3), surrounding all, a distinct basement membrane. Outside the basement membrane is an investment of connective tissue, occasional muscle fibres, and chlorogogue cells. The pharynx, on the contrary, is a more highly specialized structure. Its wall is seen in cross section (Plate **4**, Fig. 18) to consist of a dorsal arched portion with a very thick wall, a nearly flat ventral floor, and much thinner latero-ventral connecting regions. The thin latero-ventral portions of the wall may be evaginated to form a pair of longitudinal grooves (*sul.*). The flexibility of this region allows considerable variability in the form of the tube, for the floor may be infolded into the arched dorsal portion so as nearly to obliterate the lumen, or it may be depressed until the lumen is nearly circular in cross section. Strongly ciliated, long columnar epithelial cells form the dorsal wall, and extend ventrally somewhat more than half way down the sides (Fig. 18, *phy. d.*). The ventral floor (*phy. v.*) is similarly formed of ciliated cells, but these are not so long as those of the dorsal wall, while the wall of the groove is formed of cubical non-ciliated epithelial cells. The new pharynx, formed as it is during budding, is derived exclusively from material lying anterior to the dissepiment which originally marks the posterior boundary of the budding zone. This shows that it must be either a modification of the posterior half of the intestine of the segment which is involved in the budding process, or a new structure, the material for which is supplied from other growth centres within the half-segment.

In a budding worm, while the body wall of the bud zone increases in length by the rapid multiplication of the elements making up that wall, there seems to be no corresponding growth on the part of the cells of the intestine. The digestive tube is thus mechanically stretched; this tends to obliterate the inequalities of its calibre. (Compare Fig. 5 with Fig. 16.) By the continuation of this stretching, the individual cells are transformed from their original columnar character into relatively thin pavement-like elements. I have no evidence that these ciliated epithelial cells divide during the budding process.

The first signs of cellular activity in the entoderm are seen in the small, indifferent, sub-epithelial cells (Plate **2**, Fig. 13). These become more and more numerous, first in the ventral part of the gut, and later in its lateral and dorsal walls; they form at length a sheath of cells around the epithelial layer which extends through the larger part of the

active segment, and is especially well marked in its posterior half (Plate 3, Fig. 16). This cell multiplication may continue in certain places until the layer *en'drm.*^{2} becomes five or six cells deep (Figs. 7–9). The cytoplasm of the older layer (*en'drm.*^{1}) stains feebly and diffusely, while that of the new layer (*en'drm.*^{2}) takes a very deep stain. The new entodermal layer attains a greater thickness in the posterior than in the anterior zoöid. The dorsal portion of the digestive tube of the posterior zoöid, beginning just behind the brain and extending to the posterior limit of the budding zone, is the region of greatest thickening. The thickness diminishes gradually from the dorsal to the ventral side of the tube. Later a separation of the old from the new entoderm occurs, and a cavity — crescent-shaped in cross section, the lumen of the new pharynx — appears (*phy. lu.*, Figs. 9, 16). The new entodermal cells next take on the typical columnar form and then become ciliated (Plate 3, Fig. 17; Plate 4, Fig. 19). The action of the cilia is manifest before the separation of the zoöids, and before the rudimentary lumen of the pharynx has any connection with either the old lumen or the outside world. The old entodermal lining finally becomes detached throughout the pharyngeal tract (Plate 2, Fig. 14, *en'drm.*^{1}) and is swallowed or ejected through the mouth.

It is important to note that the entodermal cells are even more completely separated into two functionally different regions, corresponding to the two zoöids, than are the cells of the ectoderm. There is a distinct neutral zone between them, embracing the plane of division. This interruption is most conspicuous dorsally and laterally, being only slight ventrally (Plate 3, Fig. 16).

In the anterior zoöid, immediately in front of the future plane of separation, two pairs of thickenings arise in a manner entirely analogous to that just described for the pharynx of the posterior zoöid (Plate 3, Figs. 16, 17; Plate 4, Fig. 23; Plate 5, Figs. 24, 25, *pav.*), and in these thickenings spaces are formed, as in the case of the pharynx. Ultimately the dorsal and ventral cavities on the same side coalesce, and likewise the right and left communicate across the median plane, both dorsally and ventrally, thus forming a complete annular space of varying calibre around the old tube. These new entodermal cells, like those of the pharyngeal wall, bear cilia; they constitute the lining of the pavilion. The ectoderm has no part in forming the inner face of the pavilion, the concrescence of the free margins of the two layers, ectoderm and entoderm, taking place at the margin of the pavilion. The digitiform process, however, is wholly covered with ectoderm, the boundary be-

tween ectoderm and entoderm being at the base of the inner side of that appendage.

It is only in the region of the pavilion that the entoderm of the anterior zoöid becomes especially active during budding, but anterior to this the sub-epithelial cells form a more or less continuous layer beneath the ciliated epithelium, in a manner characteristic of the indifferent preanal zone of the mature worm. As the digestive tube lengthens in the formation of new segments, these sub-epithelial cells, in my opinion, become interpolated between the ciliate cells, and thus help to form the lining of the tube, for I find no evidence that the ciliate cells in this region are undergoing division.

Summarizing the facts concerning the growth regions in the entoderm, we may distinguish the following in the anterior zoöid: (1) the ante-pavilion region, where a single layer of sub-epithelial cells reinforces the digestive cells, the latter not being sloughed; (2) the pavilion thickening, from which the old entoderm is lost; and in the posterior zoöid, (3) the pharyngeal region, of somewhat irregular shape, surrounding the gut and extending posteriorly to the old dissepiment; from this region also the original entodermic lining is cast off. Between (2) and (3) is a neutral zone, more extensive dorsally, narrowing below, in which the old layer alone exists.

I shall deal more fully with this in discussing the mouth.

The old entoderm in the budding zone does not lose continuity until the zoöids separate; thus there is no functional mouth nor anus until separation. The cells of the original lining of the digestive tract show signs of degeneration long before separation. The cell boundaries become less distinct, the cytoplasm stains more diffusely and less intensely, and vacuoles occur in the cytoplasm.

c. Mesoderm.

The general arrangement of muscle fibres is the same in Dero as in other Oligochæta. The circular muscles lie beneath the dermis, and beneath these the longitudinal fibres are grouped in four bands: (1) the dorsal band occupies slightly more than one half the circumference of the body; (2) the narrow lateral band on either side is perhaps equal to one eighth of the circumference, and is separated by a space from the dorsal muscle; and (3) the ventral band, which is somewhat broader than the lateral bands, from which it is separated by a space.

In the formation of the budding zone the lateral bands are little interfered with until the zoöids separate, when their broken ends become

spread out and attached to the lateral walls of the pavilion in the anterior zoöid, and to the prostomium in the posterior individual. The dorsal and ventral muscle bands, on the contrary, are both split, it will be remembered, by ectodermic invasions which penetrate between their fibres. Two symmetrically located series of ingrowths penetrate the ventral muscle, so that this band throughout the bud zone is made to consist of a median (*mu. v.*) and two lateral portions (*mu. v'.*). During the ingrowth of the indifferent ectoderm tissue, the lateral parts are pushed inward much more than the median, and in the posterior zoöid they come to lie inside the circle of the newly formed pharyngeal nerve ring (Plate **1**, Fig. 6; Plate **4**, Fig. 21, *mu. v'.*). These lateral fibres of the ventral band, on account of their deeper position, are less interfered with by the formation of the ventral groove than is the median strand. As a result, they constitute the muscular connection on the ventral side of the zoöids which persists longest. Thus the mid-ventral strand is sooner free to form new attachments in connection with the mouth and head of the new individual; its fibres are in fact seen to be distributed to the lower lip, to the sides of the mouth, and to the pharynx, persisting as the principal ventral muscle. The lateral portion of the ventral band, which passes within the nerve ring, becomes attached to the deeper portion of the ectodermal constituent of the ventral surface of the mouth (Plate **5**, Fig. 26, *mu. v'.*).

In the budding zone of the anterior individual the *old* median band is superficial even to the newly forming circular muscle fibres; the band becomes broken up into smaller groups of fibres by the penetration of ectodermal elements. The deeper lateral strands of the ventral muscle band become more conspicuous, and a *new* median constituent is formed between them; in this way is reproduced the typical single ventral muscle. Anterior and posterior to the region of ectodermal ingrowth the strands merge into a normally continuous sheet.

The dorsal muscle band is likewise separated into a median and lateral portions by the development of the nerve ring, which passes exterior to the extreme lateral portions of the band (Plate **2**, Fig. 10, *mu. d'.*), but interior to the dorsal (median) part of it. Thus, while the zoöids remain connected, the brain is supported by a sling, as it were, composed of the lateral strands of the dorsal muscle bands (Plate **2**, Fig. 10; Plate **3**, Figs. 16, 17, *mu d'.*). In this position, some of the fibres become attached to the brain capsule (Plate **5**, Fig. 28); others remain attached to the dermo-muscular wall at the anterior dorsal boundary of the posterior zoöid. The contraction of the latter fibres, when separation of the

zoöids takes place, in connection with the action of the circular muscles to be described in detail later, pulls the ectodermal margin downward and backward into connection with the median-dorsal entodermal and dorso-lateral ectodermal elements previously mentioned. The median dorsal fibres are distributed to the prostomium (Plate **5**, Fig. 28, *mu. d.*).

New circular muscles are formed among the pre-existing fibres as the bud-zone increases in length. The chief special modification occurs in the immediate vicinity of the future separation. In connection with the ectodermic ingrowths contributing to the formation of the mouth, circular fibres are carried inward in such a way as to constitute an investment of the new epithelium which forms the floor of the mouth (Plate **3**, Figs. 16, 17, *mu. crc'œ.*, fibres cut crosswise).

Before separation of the zoöids, these circular fibres (Plate **5**, Fig. 25, *mu. crc'œ.*) pass, like the circumœsophageal nerve ring, between the separated strands of the ventral muscle band, a lie for and part of their course within the nerve ring. Another interesting fact concerning the circular muscle fibres of this region is that they do not constitute a complete circular band. The ends of the fibres, instead of meeting in the mid-dorsal line, pass forward as well as upward, and are inserted into the sides of the prostomium (Plate **5**, Figs. 26, 29, *mu. crc'œ.*); they aid in pulling the prostomium downward into its normal position when the posterior individual becomes detached from the anterior one. The fibres of this semicircular band (Figs. 16, 17, *mu. crc'œ.*) are coextensive, on the ventral floor of the mouth, with the latero-ventral ectodermal invaginations, and thus contribute to the buccal wall.

The radial fibres from the dermo-muscular sac to the pharynx represent longitudinal fibres, which have been diverted from their ordinary course of growth, and have become attached to the wall of the newly formed pharynx. Similarly, the fibres moving the bristle bundles are apparently derived from the muscle bands in their immediate vicinity. This is clearly true of those moving the setæ in the plane parallel with the axis of the body (i. e. the longitudinal fibres). There are also setal muscles in the transverse plane, but I am not certain as to their origin.

The dissepiments in the undifferentiated region of the anterior zoöid seem to be formed in a somewhat mechanical way. The cell proliferations of the ectoderm are, from the beginning, discontinuous. They are practically successive pairs of buds or ingrowths, which force before them the portions of the peritomal lining and its connective tissue investment which lie immediately opposite them. Between successive buds, i. e. in the septal planes, the mesodermic elements remain attached to the per-

manent ectoderm. The ectodermic buds in their further growth continue to carry the connective-tissue mesoderm before them until they come into contact with the wall of the digestive tube. The connective-tissue partition between the successive masses of ectodermic cells may be clearly seen in longitudinal sections of stages as advanced as that of Figure 16 (Plate 3), the anterior masses being more clearly separated than the posterior ones.

The blood-vessels undergo considerable branching and anastomosis in the anterior and posterior ends of the mature animal. In the part of the adult worm where budding is to take place, the dorsal and ventral vessels are not connected by conspicuous circumintestinal loops; but in the budding zone, long before separation of the zoöids, a complete vascular loop is formed in each zoöid close to the plane of division (Plate 3, Fig. 17, *vas. sng. crc.*). The complex anastomosis is not, so far as I can determine, completed until the animals become independent. The loops are of very small calibre until this occurs.

d. Summary of Changes in the Formation of the Mouth and Pharynx.

In concluding this part of the subject, it is perhaps desirable to summarize under a separate heading the chief features connected with the rather complex processes involved in the formation of the structures appropriate to the anterior end of the digestive tract. This is rendered desirable from the fact that each of the germ layers contributes to its production.

The wall of the new pharynx is formed around the old gut by a proliferation of the cells occupying the base of the original epithelium, and a delamination of the layer thus formed. The lumen of the pharynx exists, as a lacuna dorsal to the gut, between the old and new entoderm before the separation of the zoöids. Dorsally, the new entodermal wall does not reach forward beyond the position of the brain. Laterally, its anterior margin passes downward and somewhat backward, but turns forward again in the middle of the ventral side of the worm. To the curved lateral margin of this new wall is applied a pair of ectodermal ingrowths, whose history is as follows.

The groove marking the region of separation of the zoöids, relatively shallow in other regions, becomes specially deepened at two points, symmetrical to the median plane, and corresponding to the space between the ventral and lateral longitudinal muscles. The axis of these depressions lies in the plane of the groove, which inclines forward, and dorsally. The

direction of the plane of the groove is shown in Figure 3. The depth of the two infoldings is enhanced by the outgrowing digitiform appendages of the anterior zoöid. When the depressions have reached a considerable depth, they turn somewhat abruptly upward and backward on either side of the digestive tract (Plate **3**, Figs. 16, 17 ; Plate **4**, Fig. 22, *sul.*). The ectodermal wall bounding this lumen applies itself to the lateral wall of the old gut, but does not reach its mid-dorsal line. Posteriorly these ingrowths become continuous with the new pharyngeal wall described above, and ultimately, after the separation of the zoöids, their lumina, by the breaking through of the tip of the invagination, are put in communication with the lumen of the new pharynx (Fig. 19). This represents the condition attained before the zoöids separate ; the condition of the tissues involved is shown in Figures 19–24. At the moment of separation, the contraction of the semicircular muscles (Plate **3**, Fig. 17 ; Plate **5**, Fig. 26, *mu. crc'œ.*) and other muscles, both circular and longitudinal, in the posterior zoöid, pulls the whole prostomium downward, closing up the communication of the body cavity with the outside world which otherwise would result, bringing the aperture of the gut into a ventral, instead of a terminal position. Simultaneously, the lateral fibres of the dorsal muscle which lie inside the circumœsophageal commissure mechanically draw the ectoderm of the free anterior portion of the dorsal wall inward and backward, under the brain, into the space left by the failure of the lateral ectodermal ingrowths to meet in the mid-dorsal line ; thus is formed the roof of the buccal cavity. A similar service in the formation of the floor of the mouth is performed by the detached constituents of the ventral muscle, in the mid-ventral line.

The buccal cavity is thus lined with ectoderm from two sources : (1) the floor and roof are produced by a simple infolding of already formed ectoderm, unaccompanied by a process of growth ; (2) the sides, which extend farther posteriorly, by the latero-ventral ingrowths. The deep groove in the ectoderm (Plate **3**, Fig. 16, *sul.* ; Plate **4**, Fig. 23) persists as a lateral furrow at either side of the mouth (Plate **5**, Fig. 26, *i'vag. os. l.*).

When the zoöids separate, and the lumina of the lateral ectodermal invaginations become continuous with the lumen of the pharynx, there is seen in this structure a pair of grooves (Plate **4**, Fig. 18, *sul.*) which are directly continuous with the ectodermal grooves of the mouth and buccal cavity. By muscular contractions the old gut is loosened from its remaining points of attachment to the new, chiefly on the ventral aspect, and the mouth and pharynx are functionally and structurally complete.

8. Conclusions.

1. Two normal regenerative processes are to be distinguished in Dero (1) that by which new segments are formed, limited to the region immediately in front of the anal segment, and (2) the formation of the budding zone in segments which have been derived from (1). The first process gives rise to undifferentiated materials from which the bud-zone is produced; the bud-zone, in turn, produces a segment-forming zone. These two processes are so related in this species that budding may be looked upon as a specialized method of segment formation.

2. The sexual gonads in Dero vaga arise upon the posterior walls of dissepiments iii/iv and iv/v, in individuals known to be produced asexually. The dissepiment iii/iv, on which the testes occur, is produced *de novo* in the budding process, and dissepiment iv/v is the bounding dissepiment of the budding segment. These facts show a close relation between the sexual elements and the new structures formed from the indifferent cell masses during the budding process, and suggest that both are referable to the preanal segment-forming region as their source.

3. The budding zone in Dero is formed, and division takes place midway between dissepiments, i. e. in the middle of the segment, if the dissepiments mark the boundaries of segments. Semper ('76) states that in Nais barbata and Nais proboscidea budding occurs between two old segments. Von Kennel ('82) says that the bud is formed immediately behind a dissepiment in Ctenodrilus pardalis. Von Bock ('97) describes the bud as forming between segments in Chætogaster. His Fig. 14, Taf. VI. illustrates this and shows the dissepiment splitting. It appears to me, however, desirable that the method of the formation of new segments in Chætogaster be more fully made out. It seems to me that the splitting of the dissepiment may possibly be a part of the process of the formation of *new segments* rather than of the immediate production of the more specialized bud-zone, and that the separation occurs, after all, in the middle of this newly formed segment. The appearance of the gut in Figure 14 would suggest this interpretation. We know that new segments are formed in these regions in Chætogaster, and it is not clear how much of this precedes and how much follows the budding process.

4. The histological steps of bud formation in Dero agree for the most part with those described for Chætogaster by von Bock. This is true of the origin of the brain from paired ectodermic ingrowths, the reinforcement of the ventral nerve cord by serial ectodermic invasions between the longitudinal muscle bands, the ectodermic origin of the bristle sacs in the newly formed segments, the origin of the pharynx from the entodermal cells of the old gut, the paired ectodermic invaginations concerned in the formation of the mouth, and the absence of a proctodæum in the anterior individual. The chief points of difference which should be noted are as follows.

(*a.*) In Chætogaster the old entoderm and the wall of the body cavity unite directly in the anal segment. In Dero there is an outgrowth of newly formed ciliated entoderm which unites the old entoderm with the body wall. This new entoderm lines the pavilion. The anus is at the place of union of the old and new entoderm. According to von Kennel (82), there is a proctodæum in Ctenodrilus pardalis.

(*b.*) While the mouth and pharynx of Dero and Chætogaster present certain common features in their mode of origin, there are important differences between the two worms in each region. In Chætogaster the paired ectodermal invaginations ultimately run together across the median plane to the mouth, and they secure union with the new pharynx before the separation of the zoöids. The ectodermic invaginations in Dero, on the other hand, never meet, producing merely the buccal sinus at either lateral angle of the mouth cavity. The floor and roof of the mouth are formed by mechanical infolding of the appropriate portions of the anterior margin of the body wall around the old gut. This old ectoderm is brought into contact with the new entoderm and with the new ectoderm of the invaginations mentioned above, *after* the separation of the zoöids. The pharynx is formed in Chætogaster by an outgrowth of the entodermal cells on the floor of the old gut. This cell mass comes to be occupied by a lumen, single behind but continued forward as a pair of curved branches which come to communicate with the ectodermal invaginations which are the beginnings of the mouth. The old gut becomes constricted, and, together with the nerve cord and the ventral body wall lying between the fundaments of the mouth, is resorbed. In Dero the pharynx is formed around the old gut, especially well developed on the dorsal side, and the cavity of the gut becomes the lumen of the pharynx, the old wall being split away from the new entoderm, but not losing its continuity until the zoöids separate, whereupon the old wall is entirely broken down and lost. The differences cited above are perhaps to be correlated in part with the fact

that the zoöids of Dero are much less inclined than those of Chætogaster to remain associated after the development of the bud zone has gone so far as to render them capable of independent existence.

(*c.*) For Chætogaster von Bock ('97, Taf. VI. Figs. 9, 13) figures the connective as passing superficial to the lateral longitudinal muscle bands, and describes the brain as arising by the fusion of the ectodermic contributions from the spaces between the dorsal and lateral ("obere Muskellücken"), and from those between the lateral and ventral muscle bands ("untere Muskellücken"). These cell masses grow dorsad, overarching the gut, and fuse with the corresponding masses of the other side to form the brain. According to my observations on Dero, the brain is formed wholly by cells which break through the dorsal longitudinal muscle band. When the connective is formed, it lies superficial to a detached lateral portion of the dorsal muscle and returns to the body cavity between this and the lateral muscle (the region at which the cells of the lateral line occur). The regions of ectodermal activity responsible for the elements of the brain and connective are thus seen to lie immediately superficial to the lower margins of the dorsal muscle band. From this place there is an upward growth to form the brain, and a ventral growth which marks the course of the connective as it passes toward the ventral cord. The position of the mature connective external to certain longitudinal muscle fibres is thus rendered intelligible. In a similar way the region from which nervous cells spring on the ventral side, being superficial to the lateral fibres of the ventral muscle, gives rise to elements, a part of which penetrate the ventral muscle band (cutting off a lateral portion of it) to fuse with the ventral nerve chain, while others pass into the body cavity between the ventral and lateral muscles. The position of the connective in its course from the brain to the ventral ganglia is thus determined, and, as in the dorsal region, is superficial to the lateral portion of the ventral longitudinal muscle.

While it is entirely possible that the differences in the families studied may account for the apparent differences in the origin of the nervous elements, the course of the connective as figured by von Bock would suggest that the region superficial to the *lateral* muscle band is the place at which the nervous elements in this case arise, and that the more dorsal ingrowth is chiefly, if not wholly, the source of the brain, — the one between the lateral and ventral bands marking the course of the connective, rather than contributing directly to form the brain. Otherwise, it is very difficult to see why the connective should not follow the line of

least resistance along the *inside* of the body wall, rather than penetrate the body wall, as its course actually indicates. I am aware that this suggestion is based upon general considerations and upon analogy merely, while the care shown throughout von Bock's paper gives abundant ground for the trustworthiness of his results.

I have great pleasure, finally, in expressing the sense of obligation I feel to Dr. E. L. Mark, under whose direction this work has been done, for his advice and assistance throughout its prosecution.

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EXPLANATION OF PLATES.

ABBREVIATIONS.

a.	Anterior.
an.	Anus.
cl. gn.	Ganglion cells.
cl. ln. l.	Cells of the lateral line.
con't. crc'œ.	Circumœsophageal connective.
con't. tis.	Connective tissue.
d.	Dorsal.
di'sep.	Dissepiment.
ec'drm.[1]	Old ectoderm.
ec'drm.[2]	New ectoderm.
en'drm.[1]	Old entoderm.
en'drm.[2]	New entoderm.
e'th.	Epithelium.
f'br. n.	Fibrous elements of nervous system.
gn. su'œ.	Brain.
gn. v.	Ventral nerve ganglion.
in.	Intestine.
i'vag.	Invagination.
i'vag. os. l.	Lateral groove of mouth.
lu.[1]	Old lumen.
lu.[2]	New lumen.
mb. ba.	Basement membrane.
ms'drm.	Mesoderm.
mu.	Muscles.
mu. crc.	Circular muscles of body wall.
mu. crc'œ.	Circumœsophageal muscles.
mu.d.	Dorsal muscles (longitudinal).
mu.d'.	Portion of dorsal muscle detached by ectodermal ingrowth.
mu. l.	Lateral muscles (longitudinal).
mu. r.	Radial muscles.
mu. v.	Ventral muscles (longitudinal).
mu. v'.	Portion of ventral muscle detached by ectodermal ingrowth.
nph.	Nephridium.
œ.	Œsophagus.
os.	Mouth.
p.	Posterior.
pav.	Pavilion.
pav. lu.	Lumen of pavilion.
phy.	Pharynx.
phy. d.	Dorsal wall of pharynx.
phy. ec'drm.	Ectoderm of pharynx.
phy. en'drm.	Entoderm of pharynx.
phy. lu.	Lumen of pharynx.
phy. v.	Ventral wall of pharynx.
pr'c. dg.	Digitiform process.
pr'f.	Proliferation.
pr'stm.	Prostomium.
sac. drm-mu.	Dermo-muscular sac.
sb-e'th.	Sub-epithelium.
set. d.	Dorsal bristle sacs.
set. v.	Ventral bristle sacs.
sul.	Groove.
v.	Ventral.
vas. sng. d.	Dorsal blood-vessel.
vas. sng. crc.	Vascular loops.
vas. sng. v.	Ventral blood-vessel.
z.	Undifferentiated preanal zone.
z.[1]	Primary bud zone.
z.[2]	Secondary bud zone.
zo'd. a.	Anterior zoöid.
zo'd. p.	Posterior zoöid.

PLATE 1.

Fig. 1. Optical sagittal section of posterior portion of a worm not in process of budding, showing undifferentiated preanal zone (*z.*). × 30.

Fig. 2. Similar view of portion of an individual in which the bud zone ($z.^1$) is already distinguishable. × 30.

Fig. 3. Budding worm in which the secondary bud zone ($z.^2$) is recognizable. The Roman numerals indicate the number of the segment behind the prostomium of the original worm; the Arabic, those of the zoöids. The accented Arabic numerals, 1′–4′, denote the zone of undifferentiated materials out of which are to be formed the cephalic segments of the individual produced by the *second* budding. × 100.

Fig. 4. Parasagittal section of a budding segment showing ectodermal thickening and ingrowth on ventral surface only, at a fairly advanced stage. Section considerably to one side of the median plane. × 655.

Fig. 5. Parasagittal section of the whole segment at a later stage. × 218.

Fig. 6. Cross section of a stage much more advanced than the preceding, through the posterior zoöid, showing the position of the brain (*gn. su'œ.*). × 218.

Fig. 7. Transverse section of a similar stage, posterior to position of the brain, showing the regions where the ectoderm grows in between the muscle bands. × 218.

a.
prV
z
1.
a.
z
z'
2.
XVII
XVIII
pr.sdm
XIX
3.
a
p
z'
4.
pr.f
a.
set.v
sul
5.
set.d.
p
a.
en.drm.²
en.drm.¹
en.drm.pr.f
sul
6.
gn.su.oe
ant.cru.oe.
et.lu.l
sdrm
conj.tus
conj.cru.oe.
pr.f
set.v
mu.v.
mu.x.
gn.v.
7.
mu.d.
vas.sag.d.
en.drm.¹
en.drm.²
pr.f
mu.l.
mu.v.
set.v
mu.x.
pr.f

PLATE 2.

Fig. 8. Transverse section of the same worm as that from which Figure 7 is drawn, but anterior to the plane of separation. × 218.

Fig. 9. Transverse section of posterior zoöid in the region of the pharynx (*phy.*). The stage is later than that represented in Figures 6-8. The lumen appears as a cavity formed by the splitting of the old entoderm (*en'drm.*[1]) from the new (*en'drm.*[2]).

Fig. 10. Semi-diagrammatic representation of the course of the circumœsophageal connective (*con't. crc'œ.*) in its relation to the ganglia, the muscles, and the lateral line cells (*cl. ln. l.*). × 200.

Fig. 11. Parasagittal section showing the ectodermal thickening on the ventral surface of the budding segment, at a very early stage. × 500.

Fig. 12. Similar section at a later stage. × 500. (Figures 11, 12, are to be compared with Figures 4 and 5.)

Fig. 13. Part of longitudinal section of normal entodermal layer of intestine, showing sub-epithelial cells (*sb-e'th.*). × 800.

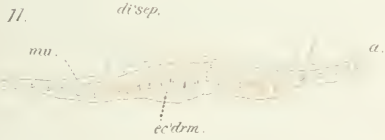
Fig. 14. Sagittal section of head of posterior zoöid which has been separated from anterior zoöid one hour, showing the extent of ectoderm and entoderm in the formation of the mouth and pharynx, in the median plane *Ec'drm.*[2] marks the boundary between new ectoderm and new entoderm; *ec'drm.*[1], the boundary between the newly formed ectoderm and the old ectoderm of the mechanically infolded edge of the prostomium, formerly attached to the anterior zoöid. × 218.

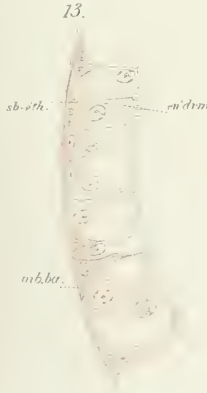
8.
mu.d
os.drm.
set.x.
mu.x.
mu.x.
ms.drm.
gn.x.

gn.su.oc
cont.orc.oe.
10.
mu.d
d.bn.l.
mu.d'
oe.
vas.sup.
mu.l.
mu.l
mu.x'
gn.x.
mu.x.

phy.lu.
phy.
en.drm.1
9.
en.drm.2
ms.drm
set.x.
prc.dg.
fbr.n.

12.
a.
sul.


11.
di.sep.
mu.
a.
ec.drm.


13.
sb.eth.
en.drm.
mb.ba.

14.
mu.r.
ec.drm.2
gn.su.oe
ec.drm.1
en.drm.1
gn.x.
en.drm.2

PLATE 3.

Fig. 15. Sagittal section showing development of the ventral nerve chain in the preanal zone of a recently separated zoöid. × 312.

Fig. 16. Parasagittal section, considerably to one side of median plane; stage about the same as that represented in Fig. 9. × 218.

Fig. 17. Similar section, later stage. × 375.

mu.d
qn.suiö
phy.lu.
phy.endrm
endrm.
16.
a
p.
perv.
shfth
phy.endrm
z'
z
sui.
pred.
vas.sug.crc.
endrm.
an.
pav.
15.
a.
ec'drm.
mu.crc.
qn.v.
fbrn.
mu.v.
qn.suiö
pr'stm.
mu.d.
mu.d'
phy.lu.
17.
pav.
endrm'
p.
a.
mu.crc'ö
qn.v
z
vas.sug.crc.

PLATE 4.

Fig. 18. Cross section of the pharynx of a mature worm. × 312.

Figs. 19–24. Posterior face of cross sections from a single series through a bud-zone in which separation is imminent. × 312. The higher the number, the farther forward the section is in the series. Figures 19–21 belong entirely to the posterior zoöid. Figures 22–24 show parts of both zoöids. Sections 10 μ thick.

Fig. 19. Section through pharynx of posterior zoöid; dorsal lumen conspicuous.

Fig. 20. Next section in front of that of Figure 19 showing anterior portion of dorsal pharyngeal lumen.

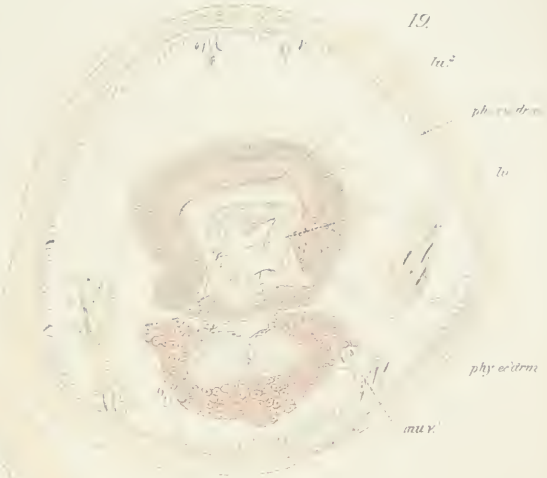
Fig. 21. Same series, two sections anterior to the preceding.

Fig. 22. Section (3rd in front of that of Figure 21) passing through ectodermal invagination (*sul.*) between the zoöids. The entodermal wall of pavilion (*en'drm.*) is seen below the gut.

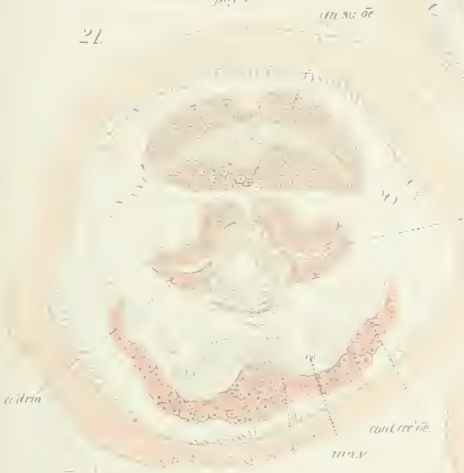
Fig. 23. The section immediately in front of the last, showing that on the left side the plane of section is farther cephalad than on the right side.

18

19



21



22

23

20

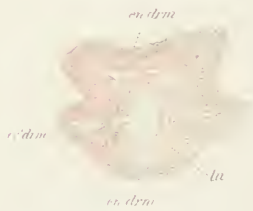


PLATE 5.

Fig. 24. Only the margin of the prostomium of posterior zoöid appears in this section, which is three sections anterior to that of Fig. 23. The ventral part of the section passes through the undifferentiated segment-forming zone of the anterior individual. (Compare Fig. 15, Plate 3, which represents a more advanced stage in longitudinal section.)

Fig. 25. Frontal section through stage of about the same age as that shown in Fig. 16, Plate 3. × 218.

Fig. 26. Frontal section through head of posterior zoöid which has been separated from the anterior zoöid two hours. × 166.

Fig. 27. Section of same series, four sections ventral to the preceding. The lateral grooves of the mouth are shown (*i'vag. os. l.*). × 166.

Fig. 28. Parasagittal section, nearly median, through the head of a posterior zoöid, showing distribution of muscles. Ventral nerve cord is not shown. × 166.

Fig. 29. Parasagittal, but more lateral, section from the same series. × 166.

26.
en.drm²
pr.stm
ivag.o.l
mu.l
mu.v
mu.cr.oe
cont.cr.oe
mu.v
gn.v
a.
pav.
cont.cr.oe
mu.v
mu.cr.oe
en.drm²
27.
pr.stm.
en.drm²
ivag.o.l
mu.cr.oe.
set.v.
VII
mu.v
VII
pr.drm
24
en.drm.
pav.tu.
mu.v
gn.v
mu.v
28.
plx.
mu.d
mu.d
gn.su.oe
mu.v
mu.cr.oe
en.drm
29
os
mu.cr.oe.

THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos.

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[3] XV.[16] The Stalk-eyed Crustacea.

S. GARMAN. The Fishes.

W. GIESBRECHT. XVI.[15] The Copepods.

A. GOËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

H. LUDWIG. IV.[5] XII.[14] The Holothurians.

C. F. LÜTKEN and TH. MORTENSEN. The Ophiuridæ.

OTTO MAAS. XXI.[21] The Acalephs.

J. P. McMURRICH. The Actinarians.

E. L. MARK. XXIV.[24] The Cerianthidæ.

GEO. P. MERRILL. V.[6] The Rocks of the Galapagos.

G. W. MÜLLER. XIX.[19] The Ostracods.

JOHN MURRAY. The Bottom Specimens.

A. ORTMANN. XIV.[12] The Pelagic Schizopods.

ROBERT RIDGWAY. The Alcoholic Birds.

P. SCHIEMENZ. The Pteropods and Heteropods.

W. SCHIMKÉWITSCH. VIII.[8] The Pycnogonidæ.

S. H. SCUDDER. VII.[7] The Orthoptera of the Galapagos.

W. PERCY SLADEN. The Starfishes.

L. STEJNEGER. The Reptiles.

TH. STUDER. X.[10] The Alcyonarians.

C. H. TOWNSEND. XVII.[17] The Birds of Cocos Island.

M. P. A. TRAÜTSTEDT. The Salpidæ and Doliolidæ.

E. P. VAN DUZEE. The Halobatidæ.

H. B. WARD. The Sipunculoids.

H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June 1891, 16 pp. ; and Vol. XXIII., No 1, February, 1892, 89 pp , 22 Plates

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z , Vol. XXV , No 1 September, 1893, 25 pp.

[8] Bull. M. C. Z , Vol. XXV., No. 2, December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4, January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C Z , Vol. XXV No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV. No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C Z , Vol XXV. No. 10, October, 1894, 109 pp , 12 Plates.

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[24] Bull. M C. Z., Vol XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, July 1899, 4 pp., 1 Plate.

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Bulletin of the Museum of Comparative Zoölogy
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Vol. XXXV. No. 6.

THE PHOTOMECHANICAL CHANGES IN THE RETINAL PIGMENT OF GAMMARUS.

By G. H. Parker.

With One Plate.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
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No. 6. — *The Photomechanical Changes in the Retinal Pigment of Gammarus.*[1] By G. H. Parker.

The following studies were made with the intention of comparing the photomechanical changes in the retina of one of the simpler crustaceans with those already known in the decapods. In this respect, very few of the simpler forms have been studied, and almost all that has been done is contained in a single paper by Szczawinska ('91). This paper appeared shortly before Exner's ('91) well known monograph, in which the photomechanical changes of the compound eye first received a consistent physiological interpretation, and consequently it does not touch several important questions raised by Exner's work.

The lower crustaceans studied by Szczawinska were Gammarus, Phronima, and Branchipus, and of these, judging from the figures given, Gammarus has the most pronounced photomechanical changes. I have therefore studied the common American form, Gammarus ornatus Milne-Edwards. Vigorous individuals of this species were kept, some in the dark and some in the light, for six hours, and were then killed by being momentarily immersed in hot water (85° C.). They were afterwards cut in sections, and their eyes studied and compared.

The structure of the eye in Gammarus ornatus has already been described (cf. Parker, '91; p. 68), and agrees almost exactly with that of G. pulex as given by Carrière ('85, p. 156). Szczawinska's ('91, p. 534) description of the eye in G. roeselii is so different from the accounts given for the other two species that I am persuaded it must be in part erroneous.

In G. ornatus the corneal cuticula (Fig. 1, *crn.*), which is not facetted, is covered internally by a corneal hypodermis (*cl. crn.*) the cells of which are not regularly grouped with reference to the ommatidia. The axis of each ommatidium is occupied distally by a two-celled cone (*con.*) and proximally by a slender rhabdome (*rhb.*). These two structures are entirely distinct from each other and not continuous, as described by

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College. E. L. Mark, Director. No. C.

Szczawinska ('91, p. 536). The transparent axis thus formed is sheathed laterally by five elongated pigment cells, the retinular cells (*cl. rtn.'*). Each retinular cell consists of three parts: a flattened distal portion applied to the side of the cone; a middle rod-like portion (Fig. 2, *cl. rtn.'*) lying against the rabdome; and an enlarged basal portion proximal to the basement membrane (*mb. ba.*) and containing the nucleus. The proximal end of this last part becomes attenuated and forms a retinal nerve fibre (*fbr. r.*) which passes to the optic ganglion. All parts of these cells except the nucleus may contain more or less blackish pigment. The space between the ommatidia is filled with a coarse granular pigment, whitish by reflected light and containing nuclei (*nl. sn.*). This material is made up of the accessory pigment cells, the boundaries of which are not easily discernible.

In Szczawinska's description of the eye in Gammarus roeselii, the three parts of the retinular cells described above are stated to be each a separate cell containing its nucleus. She correctly identified the nucleus in the proximal portion. What she believed to be the nuclei of the middle parts (Planche XVI. Fig. 4, *n.²pg.*) are without question the nuclei of the accessory pigment cells, which she failed to recognize as such. In the distal portions of the retinular cells of Gammarus ornatus nothing resembling nuclei could be discovered, and I am of opinion that she was mistaken in attributing such bodies to the corresponding parts in G. roeselii. The continuity of the three parts of the retinular cells in G. ornatus can be clearly demonstrated in serial transverse sections, in longitudinal sections, and in isolation preparations, and I therefore believe that these three portions are only parts of one cell. If this is true of G. ornatus, it is probably also true of G. roeselii, and I am strengthened in this belief as Szczawinska's own figures (Planche XVI. Figs. 4, 5) admit more readily of this interpretation than they do of her own. In these respects, then, her account is probably at fault.

In an eye of G. ornatus that had been subjected to light for some six hours (Fig. 1), a considerable amount of black pigment was found uniformly distributed through the distal and middle portions of the retinular cells, thus sheathing the cone and rhabdome laterally (Fig. 2). The proximal portion of each cell contained a few irregularly scattered pigment granules except near the nucleus, where the pigment was more abundant.

In an eye from an animal kept some six hours in the dark (Fig. 3), the pigment in the distal portions of the retinular cells presented the same condition as in the eyes exposed to light. The middle portions,

however, were almost entirely devoid of pigment, while the proximal portions were as densely filled with pigment as the distal portions.

Obviously the changes induced by the presence or absence of light affect the pigment of only the middle and proximal parts. When an animal that has been kept in the dark is exposed to the light, the pigment that is massed in the proximal parts of the retinular cells (Fig. 3) migrates distally and fills the middle portions, without however entirely abandoning the proximal parts, especially around the nucleus (Fig. 1). When an animal that has been kept in the light is placed in the dark, the pigment in the middle portions (Fig. 1) migrates into the proximal parts till almost no pigment is left in the middle portions. In other words, the presence of light induces a distal migration of much of the pigment from the proximal parts and the absence of light brings about a proximal migration of almost all the pigment in the middle parts.

In none of my observations was there any evidence of photomechanical changes in the accessory pigment cells.

Aside from the disparity due to the different anatomical descriptions of the eyes, the physiological results given in this paper confirm in the main those given by Szczawinska ('91, p. 548). In one respect only is there a significant difference. Szczawinska claims that in G. roeselii the pigment in what I have called the distal parts of the retinular cells shows photomechanical changes. In G. ornatus no evidence of such changes could be found, and since in G. roeselii, according to the figures given by Szczawinska (Planche XVI. Figs. 1, 2), the supposed evidence of these changes may be entirely the result of a slight difference in the planes at which the sections have been cut, it may fairly be doubted if these changes occur at all.

The relations that the photomechanical changes, described above, bear to the physiology of the eye in G. ornatus are not far to seek. Light passing through the axis of any cone in the eye of this animal would be conducted directly to the rhabdome under the given cone. Light entering a cone obliquely to its axis would fall upon one of its pigmented sides, where, if not absorbed, the light would suffer reflection. As the sides of the cone are not parallel but approach proximally, the light would not undergo simple internal reflection as in a cylinder, but would be so turned at each reflection that it would eventually be discharged from the end of the cone at which it entered. Thus oblique light would not reach the underlying rhabdome at all. This action, by which the axial light of the cone is conducted to the rhabdome and the oblique light is discharged, has been called by Exner ('91, p. 59) the catoptric action of

the cone, and has been fully described by him. In accordance with this relation, then, the rhabdome of each ommatidium would receive light from an external region corresponding to the outward projection of the axis of the cone distal to it.

As the pigment which surrounds the cone is merely concerned with the absorption of the lateral rays, and as these rays would be equally disturbing whether the animal were in bright light or dim light, it follows that no photomechanical changes in correspondence with changes in the intensity of the light should be expected in this pigment, and as a matter of fact in G. ornatus no such changes have been observed.

The axial light, which according to the foregoing account finds its way into the rhabdome, must have a certain degree of intensity in order to stimulate that organ. Ordinary daylight is presumably more than sufficient to call forth this stimulation, and such superfluous light as may pass to the edges of the rhabdome or through it is probably absorbed by the black pigment that in bright light (Figs. 1, 2) surrounds that body. In dim light, however, there must be times when the light which enters the rhabdome is scarcely intense enough to stimulate that organ. Under such circumstances the more oblique rays, which ordinarily would be absorbed by the black pigment on the sides of the rhabdome, would materially aid in stimulating it if they were turned back into the rhabdome. That these rays are probably thus turned back is shown by the fact that in dim light the black pigment is removed from the rhabdome and the surrounding whitish reflecting pigment of the accessory pigment cells is exposed (compare Figs. 2, 4).

Changes exactly comparable with these have been described in the proximal retinular cells of the higher crustaceans. The changes in the distal retinular cells, the iris pigment of Exner, which are connected with the formation of superposition images, find no representatives in the eyes of Gammarus ornatus. As this eye is in many respects primitive, it is likely that the ancestral crustacean ommatidium possessed a catoptric cone and a retinula provided with a reflecting apparatus for use in dim light. In the differentiation of the higher type of crustacean ommatidium the reflecting mechanism was retained, but the catoptric cone gave way to a second type of cone provided with special pigment cells whose movements were associated with the superposition images formed by this type of cone.

Summary.

1. In Gammarus ornatus photomechanical changes in the retinal pigment are not observable in the accessory pigment cells or in the distal parts of the retinular cells, but are limited to the black pigment in the middle and proximal portions of the retinular cells.

2. In the light the middle portion of each retinular cell is well filled with pigment, thus enclosing the rhabdome in a black sheath; the proximal portion contains scattered grains except near the nucleus, where the pigment is more massed.

3. In the dark the middle portion of each cell is almost free from pigment, which now fills the proximal part.

4. In the dark the removal of the black pigment from around the rhabdome exposes the accessory pigment cells, which probably act as reflecting organs, and in very dim light turn such rays as have escaped laterally from the rhabdome back into that structure, thus aiding in an effective stimulation of this organ.

Cambridge, December 15, 1898.

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EXPLANATION OF THE PLATE.

All the figures were taken from preparations of the eyes of Gammarus ornatus M.-Edw. They were drawn with the aid of an Abbé camera, and are magnified 570 diameters.

ABBREVIATIONS.

cl. crn.	Corneal hypodermis.	*fbr. r.*	Retinal nerve fibre.
cl. rtn.'	Retinular cell.	*mb. ba.*	Basement membrane.
cl. sn.	Accessory pigment cell.	*nl. rtn.'*	Nucleus of retinular cell.
con.	Cone.	*nl. sn.*	Nucleus of accessory pigment cell.
crn.	Corneal cuticula.	*rhb.*	Rhabdome.

Fig. 1. Longitudinal section of an ommatidium, showing the arrangement of pigment due to exposure to bright light.

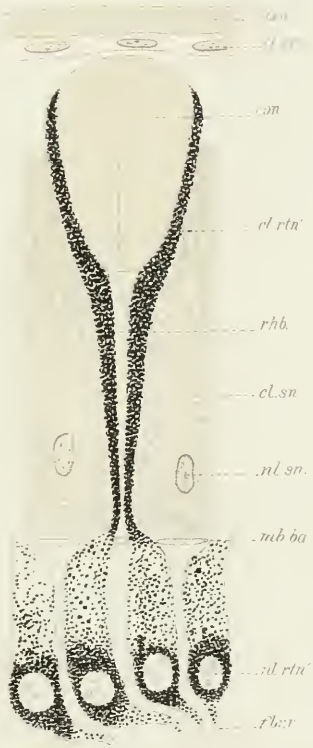
Fig. 2. Transverse section of a retinula from such a preparation as that shown in Figure 1, and taken near the level marked *rhb.* in that figure.

Fig. 3. Longitudinal section of an ommatidium showing the arrangement of pigment due to the absence of light.

Fig. 4. Transverse section of a retinula from such a preparation as that shown in Figure 3, and taken near the level marked *rhb.* in Figure 1.

LIGHT

DARK.



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2.

4.

THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

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A. GOËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

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W. E. HOYLE. The Cephalopods.

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R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

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E. P. VAN DUZEE. The Halobatidæ.

H. B. WARD. The Sipunculoids.

H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

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[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

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Bulletin of the Museum of Comparative Zoölogy
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VOL. XXXV. NO. 7.

THE STRUCTURE AND DEVELOPMENT OF THE ANTENNAL GLANDS IN HOMARUS AMERICANUS MILNE-EDWARDS.

BY FREDERICK C. WAITE.

WITH SIX PLATES.

CAMBRIDGE, MASS., U. S. A.:
PRINTED FOR THE MUSEUM.
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No. 7. — *The Structure and Development of the Antennal Glands in Homarus americanus Milne-Edwards.*[1] By Frederick C. Waite.

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Introduction.

The antennal glands of Crustacea have been the subject of much discussion ever since the first discovery of them in Astacus by Rösel von Rosenhof (1755, p. 321). The true relation of the parts of the organ was for a long time overlooked, and the gland proper was considered as distinct from the storage reservoir and duct to the exterior. The former was supposed to be connected with the digestive tract, either as a salivary gland or as the source of the gastroliths, while the latter was taken for a sense organ, first as auditory, later as olfactory. Brandt ('33, p. 64) showed that the parts which had been considered as two organs of different functions really constituted a single structure. To this was ascribed the function of hearing.

All observations were confined to Astacus until Semper ('61) extended his work to Lucifer. Shortly afterward Claus ('63) added observations

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. 105.

upon a few other forms. The first extensive comparative work was that of Grobben ('80), who studied these organs in a number of Entomostraca and Malacostraca, and sought to prove them homologous in the two groups.

The earliest evidence of the excretory function of these organs was afforded by Will und Gorup-Besanez ('48), who made the following statement (column 828): ". . . in der That haben wir im sogenannten grünen Organ des Flusskrebses (Astacus fluviatilis) und im Bojanus'-schen Organ des Teichmuschel (Anodonta) einen Stoff aufgefunden, der Reaktionserscheinungen zeigte, die mit der grössten Wahrscheinlichkeit auf Guanin hinweisen, doch gebrach es uns bisher an dem nöthigen Material, um entscheidende Versuche damit anzustellen." Similar chemical experiments were carried on by several later investigators, but the most conclusive evidence of the excretory activity of these organs rests upon extensive experiments in *intra vitam* injection and feeding which were inaugurated by Kowalevsky ('89), and extended in subsequent papers by him and by other authors, especially by Cuénot ('94).

By far the most comprehensive work on excretion in Crustacea is that of Marchal ('92). Until the publication of this work, the major part of the literature, especially as regards histology, was on Astacus, with additions on some of the Caridide (Grobben '80, Kowalevsky '89, and Weldon '89 and '91). Marchal in his memoir gives the results of a careful research on the morphology, histology, and physiology of a long series of species representing all the typical genera of the Decapoda. The work was carried on by the methods of modern technique, including the *intra vitam* method of Kowalevsky ('89).

This is the only paper that deals with the antennal gland in Homarus. The species studied by Marchal (H. vulgaris), however, was not the same as that of the present paper. In H. vulgaris, according to Marchal (pp. 156–163), the gland presents an entirely different appearance from that seen in Astacus, although the main divisions into gland proper, vesicle, and duct are found in both. In H. vulgaris the gland as seen from above is triangular in shape, the antero-posterior axis being the longest. The posterior tip is curved mediad, and the periphery is more or less notched. The dorsal face is slightly concave, the ventral markedly convex.

The structure is much less complex than in Astacus, and there are two distinct regions besides the duct, — the saccule and the labyrinth. The former is thin and flattened out over the dorsal face of the gland, covering it except for a narrow projecting margin. The saccule has a

large central cavity, from which radiate ramifying culs-de-sac. The labyrinth is three times as thick as the saccule and forms the greater bulk of the gland. It is divided by an antero-posterior fissure on the ventral face, so as to be U-shaped, the arms of the U pointing anteriorly. The connection with the overlying saccule is at the end of the lateral arm, that with the excretory duct at the end of the median arm. In the latter case, this is by means of several parallel canaliculi, which open as pores in the wall of the duct. The vesicle is a dorsal outpocketing from the duct, and is not in direct connection with the gland. The labyrinth consists of a spongy tissue formed by the walls of innumerable canals, which anastomose with one another without any determinable system.

The cells of the saccule are narrow, crowded, with rounded free ends, and without cuticula, while in the labyrinth the cells are markedly striate, and surmounted often by clear vesicles. A cuticuloid covering is sometimes present.

The present state of opinion as to the function of the antennal glands in Decapoda is not one of complete accord. It needs but a superficial examination of the epithelium in these organs to convince one of their glandular nature. The *intra vitam* experiments of Kowalevsky ('89) and Cuénot ('94) show conclusively that the cells take up grains of coloring matter introduced into the circulation and eliminate them. Moreover, with the exception of the ductless branchial glands and certain cells of the liver, this function is confined, as far as we at present know, to these antennal glands. The glandular secreting cells of the intestine perform no such function. Conclusions like those of Will und Gorup-Besanez ('48), who asserted the presence of guanin, have been reached by H. Dohrn ('61),[1] by Kirch ('86), and especially by Griffiths ('85). Griffiths got definite tests for uric acid, and he carefully describes the chemical analysis by the murexid method; further, he obtained small traces of the base guanin. Szigethy ('84) also found what he believed to be uric acid crystals. In each of these cases the analysis was upon the gland in Astacus.

Marchal ('92, pp. 237–245), on the other hand, made analyses of the excremental fluid of Maia squinado, and could get no traces of urea or uric acid, but did obtain nitrogen. He however obtained an acid somewhat analogous to uric acid, which he terms carcinuric acid. This be-

[1] I quote Dohrn and Kirch on the authority of Gerstaecker ('95, p. 1009), as the original papers are inaccessible to me.

longs to the series of the carbopyridic acids. There is also found a base, leucomaine, which is analogous to the vegetable alkaloids. He denies that the gland in Astacus contains uric acid.

It is difficult to reconcile these diverse results. It is undoubtedly true that the antennal glands eliminate certain ammonia compounds, and it is also shown by Marchal ('92, p. 243), that one of their functions is to rid the organism of the excess of mineral salts present in the blood, and Kirch ('86) has found that in Astacus these organs contain glycogen. Gerstaecker ('95, pp. 1009, 1010) concludes that, in view of all these results, we must consider the antennal glands of Decapoda not as closely analogous to a kidney, but as excretory organs important in the general metabolism; and, by reason of recent work, that we must concede the physiology of the antennal glands to be in many respects an open question, the solution of which will require much research, and that it is not wholly improbable that these glands, like the liver, are subject to temporarily changing functions.

This seems a fair and conservative statement, which expresses the present state of our knowledge in regard to the function of these organs.

The work represented by this paper was done during the years 1896–97 and 1897–98 in the Zoölogical Laboratory of Harvard University. During the year 1896–97 my work was aided by privileges afforded by a Morgan Fellowship in that University. I wish here to express my indebtedness to Prof. E. L. Mark, Director of the Laboratory, for his kindness in many ways, and for his supervision and able criticism of the work during its progress. I am also indebted to Mr. Alexander Agassiz for the opportunity of working at his Newport Laboratory during part of the summer of 1897, and for appointment to a table at the United States Fish Commission Laboratory at Wood's Hole during the summer of 1896; to Hon. J. J. Brice, formerly United States Commissioner of Fish and Fisheries, for courtesies extended at various times at the Wood's Hole and Gloucester stations of the United States Fish Commission; and to Prof. F. H. Herrick and Dr. G. H. Parker for the use of embryonic material of certain stages.

Methods.

The methods employed were different in the embryonic, larval, and adult stages.

In studying the finer anatomy of the adult organ, I have had recourse to maceration, teasing, and serial sections. For sectioning, the

most serviceable fixing reagents were, in the order named, the chromosmo-picro-acetic mixture of vom Rath followed by pyroligneous acid, Perenyi's fluid, and corrosive sublimate. For staining the material fixed in Perenyi's fluid and corrosive sublimate, I have used Heidenhain's iron hæmatoxylin, Delafield's hæmatoxylin, and Ehrlich's acetic acid-alum hæmatoxylin. For a double stain orange G of Grübler after either of the last two is of value. In general the carmines are not serviceable in this connection, but picro-lithium carminate has afforded very good results on the wall of the vesicle.

Both surface preparations and serial sections were used in studying embryos. The yolk was sometimes dissected away before sectioning, but at other times it was sectioned with the embryo. The selection of the killing agent to be used depends in a measure upon which of these two methods is to be followed in sectioning. The specimens to be used for examination by transmitted light must necessarily be separated from the yolk. This was accomplished with fine needles and a small stream of fluid from a pipette, the work being done under a dissecting microscope and in alcohol of a lower grade than that in which the embryos had been kept. This change in the grade of alcohol distends the egg membrane so that it may easily be shelled off. The difficulty of the separation of the yolk depends upon its friability and the age of the embryo.

I have used for killing the following: (1) water, heated to boiling and then quickly poured over the embryos; (2) saturated aqueous solution of corrosive sublimate, used either hot or cold; (3) Perenyi's fluid; (4) Kleinenberg's picro-sulphuric mixture; (5) Flemming's fluid, — weaker mixture. Of these the hot water and the corrosive sublimate treatment render the yolk granular and friable, so that it is easily separated from the embryo; at the same time the yolk particles are so loosely united that it is impracticable to section embryos with the yolk attached. On the other hand, treatment with Perenyi's fluid makes the yolk so firm and tough that it is almost impossible to dissect off the embryo without serious injury to it. Treatment with picro-sulphuric mixture, if of short duration (15 to 30 minutes), allows the yolk to be removed in large masses, and completely, without injury. Longer treatment (60 to 90 minutes) interferes with such removal, but solidifies the yolk so that it can be easily sectioned with the embryo. The weaker Flemming's fluid when used for a short time (20 to 30 minutes) gives a tenacious rind enclosing the embryo, which is easily separable from the coarsely granular central part of the yolk. A longer treatment (45 to 90 minutes) gives the best condition I have obtained for sectioning

entire the yolk and embryo. The time of exposure to any killing fluid should vary with the age of the embryo.

I have tried a variety of stains. Of the many carmine combinations used, I have found Grenacher's aqueous alum carmine the most serviceable. The best results are gained from material killed with hot water. Of the hæmatoxylins, I have found Ehrlich's acetic acid-alum mixture as good as any, and the most reliable. It stains material fixed by any of the methods mentioned, the best results being obtained after fixing with Perenyi's fluid or with Flemming's weaker mixture. I have found, however, that the staining is much better if the hæmatoxylin is followed by an aniline dye. Of several tried I have found orange G of Grübler much the best; consequently I have used this excellent combination in most of my work. The results are precise and clear, and the manipulation of staining is very simple. Sections $6\frac{2}{3}$, 10, or $13\frac{1}{3}$ micra in thickness are stained on the slide in Ehrlich's mixture from 20 to 30 minutes, then washed in acidulated alcohol until they are very pale, next neutralized in a two per cent solution of bicarbonate of soda, then thoroughly washed in distilled water and put into a saturated aqueous solution of orange G. This is allowed to act from 10 to 20 minutes, the time being determined by watching the action of the stain. The slide, after draining, is transferred directly to absolute alcohol, which sets the stain and at the same time washes out the excess. After clearing in xylol, the sections are mounted in xylol balsam, the stain being thoroughly permanent.

In studying the larval organ I have depended entirely upon serial sections, for the gland is too small to permit satisfactory dissections, and the shell of the appendage does not clear readily enough to permit study of the entire organ *in situ*. The first larva was obtainable in abundance, so that various methods could be tried, but the older larvæ are more difficult to get, and as a matter of fact all of the older larvæ which I had were already preserved when they came into my hands.

The most successful results with the first larva were obtained with the weaker Flemming's mixture, Perenyi's fluid, corrosive sublimate, or Kleinenberg's picro-sulphuric mixture. I give these reagents preference in the order named; the last, however, is not available in the older larval or the adolescent stages, when the shell comes to contain considerable quantities of lime salts.

For this stage several stains have been used. I finally settled upon Ehrlich's acetic acid-alum hæmatoxylin, however, as the most reliable stain for the gland. For a double stain I have used this followed by acid fuchsin or orange G, the latter being, in my opinion, preferable.

The paraffin method of embedding has been used exclusively. The cuticular shell in the larval stages is rather resistant, and soon dulls the knife, but with a sharp knife good series $6\frac{3}{4}$ micra in thickness were obtained.

I. Structure in the Adult.

The structure of the adult antennal gland in the genus Homarus has been described by only one writer, Marchal ('92, pp. 156–163, Plate VII. Fig. 1), who gives an account of the organ in H. vulgaris. There is no published account of this gland in H. americanus except a short abstract of this paper (Waite, '98).

A. Gross Anatomy.

The gland, with its accessory structures, is situated at the base of the second antenna, and the most of it is within the cephalothorax. It occupies the greater part of the space beneath, and on the side of the masticatory stomach, anterior to the voluminous hepato-pancreas. The organ as a whole may be divided roughly into three parts, which are easily recognized and sharply separated, namely: (1) the gland proper, often referred to hereafter simply as the gland; (2) the overlying vesicle, or storage reservoir; and (3) the duct leading to the exterior, together with the tubercle upon which it opens.

The *gland proper* lies almost entirely on the ventral floor of the cephalothorax, but its anterior median lobe extends into the base of the antenna. It lies close to the sagittal plane, the median edges of the two glands being but about five millimetres apart.[1] None of the principal axes of the gland proper are parallel to the principal axes of the body. The dorsal face is so inclined to the frontal plane of the animal that its anterior edge is 20° or 30° more dorsal than its posterior edge, and its lateral border 25° or 30° more dorsal than its median. The chief axis of the gland as viewed from above runs from the anterior notch or hilus to the most distant point of the posterior edge (Plate **1**, Fig. 1). This axis makes an angle of about 30° with the sagittal plane of the animal, its anterior end being nearer that plane.

The general shape of the gland proper as seen from above resembles that of a cordate leaf with a blunt point, the notch being anterior (Plate **1**, Fig. 1). The dorsal face is irregularly concave. This concavity is filled

[1] All measurements given for the adult gland are for an average adult nine inches (= 23 cm.) in length from tip of rostrum to tip of telson.

by the ventral part of the overlying vesicle, the wall of which is closely applied to the smooth dorsal surface of the gland (Plate **1**, Fig. 2, *par. vs.*). The ventral face of the gland is in general convex, but has in it depressions which conform to the underlying hard parts of the endophragmal system and shell, and to the muscles upon which the gland rests. The ventral surface differs much in appearance from the smooth dorsal surface. It is uneven, being thrown into numerous ridges and furrows, resembling to some extent the gyri and sulci of the cerebral hemispheres in higher mammals. All these furrows radiate in a general way from a region of the gland lying near the bottom of the hilus, but the individual furrows are comparatively short, and separated by ridges from other furrows in the same radius. The furrow lying in the main axis of the gland is the most prominent and deepest one. It is a direct continuation of the hilus. The dorso-ventral diameter varies, but is greatest in the centre of the organ, and thins out to a translucent edge on the borders. The outline of the border of the gland is irregular. In the middle of the anterior edge there is a sharp V-shaped hilus extending posteriorly 2 to 3 millimetres (Plate **1**, Fig. 1, *hi.*). The bottom of the hilus marks very nearly the centre of the gland. The median edge of the gland presents a simple curve convex toward the median plane of the body, and having a radius of about a centimetre. The lateral edge is S-shaped, the posterior bend of the S giving the gland the appearance of being notched to accommodate the flexor antennarius muscle, on which it does in part rest. This notch is 2.5 or 3 mm. deep and 5 or 6 mm. broad. The edge of the gland for the most of its extent is bluntly toothed or lobed, so that the outline is more or less sinuous.

For purposes of description the gland proper may be said to present three lobes: a median anterior (*lob. m-a.*, Plate **1**, Fig. 1), a lateral anterior (*lob. l-a.*), and a posterior one (*lob. p.*).

The gland measures in its longest diameter, from the edge of the median anterior lobe to the posterior apex, 13 to 15 mm.; from the bottom of the lateral notch to the middle of the median border, 9 to 10 mm. Its greatest dorso-ventral thickness is about 3 mm. It is thus evident that the gland is much flattened.

In color the gland is a light olive, the ventral face being of a darker shade than the dorsal; the latter in some individuals approaches yellowish brown. The light dorsal surface is surrounded by a narrow border of the darker shade from 1 to 2 mm. in width (Plate **1**, Fig. 1, *marg.*), which is left without tint in the figure. This border widens to quite a broad band on the median anterior lobe. The area of lighter

color corresponds with the extent of the endsac; that of the darker border, with that part of the dorsal face of the labyrinth which is not covered by the endsac, or which shows through the attenuated edge of the endsac.

From the median anterior lobe there is given off a smaller lobe 2.5 to 3 mm. in diameter and 5 to 6 mm. in length. This is directed caudad and ventrad, and tapers to a point. Its general shape is conical. In color it is so distinct from the other parts of the gland that Marchal ('92, p. 159) has called it the white lobe. The tapering point opens into the excretory duct in a region ventral to the centre of the gland. This opening is not single, but consists of several minute pores, to each of which there leads one of a series of converging tubules which are in turn formed by the confluence of other smaller tubules in the basal part of the white lobe. These pores are on the dorso-median wall of the duct, 2 to 3 mm. from its external orifice. By these the products of the gland are discharged into the duct. *This is the only communication which the gland proper has with its accessory structures,* there being no direct communication between the gland and the vesicle, as there is in Astacus. Therefore the vesicle must be filled by the backing up of the secreted products in the duct from the point where the pores open, a process which is presumably effected by closure of the external opening of the duct.

The *vesicle* is situated in the cephalothorax at the side of the masticatory stomach. Its anterior end lies against the muscles of the body wall. On its median face it presses against the stomach, while posteriorly it reaches the hepato-pancreas and extends between its lobes. Ventrally, it is in part closely and firmly attached to the dorsal face of the gland (Plate 1, Figs. 1, 2, *par. vs.*). Posterior to the gland it lies free upon the floor of the cephalothorax. Its shape is roughly oval, its long axis lying in a parasagittal plane. Usually the posterior end is slightly divided into two blunt lobes. Since the thin elastic walls easily conform to the surrounding organs, its actual shape in detail is irregular, depending upon the state of its own distention and that of the stomach, hepato-pancreas, and reproductive organs, and the contraction or relaxation of the muscles in this region. In some individuals it seems to fill every niche of unoccupied space; in others there are considerable spaces between it and the body wall.

I have found no ligamentous attachments for holding the vesicle in place other than that to the dorsal surface of the gland, already noted, and those to the blood-vessels and nerves which come to its surface.

The wall of the vesicle is uniformly very thin and transparent in all parts except immediately around the orifice of the duct, where it is somewhat thicker; but there is no muscular sphincter at this point. The histological structure of the wall is described in another connection (pp. 166, 167).

At the anterior end of the vesicle, at a point on a line with the median border of the hilus and a little in front of the anterior edge of the gland, there is an orifice which opens into the duct leading to the exterior (Plate 1, Fig. 1, *of. i.*). This orifice is circular, or slightly oval, and 1 to 1.5 mm. in diameter. It is noticeable that it retains its shape when the walls of the vesicle are collapsed.

The *duct* is a thin-walled tube, oval in cross section, — being slightly flattened dorso-ventrally, — and lies wholly within the antenna. From the orifice in the vesicle it extends downward and slightly forward and outward for a distance of 5 to 7 mm. Its average diameter through this region is 2 mm. At the level of the coxo-basal joint the duct turns abruptly through an arc of about 45° in a parasagittal plane of the body, and runs downward and backward to the tubercle on the ventral face of the coxopodite, through which it opens to the exterior. Its diameter through this second part of its course is somewhat greater than in the first part, but diminishes noticeably as it enters the tubercle. There is a slight dilatation at the point where the tubules from the white lobe enter. The entire length of the duct is not over 1.5 cm., and its average diameter is perhaps 2.5 mm.

I find no sphincters or other muscular structures in or around the wall of the duct, other than the muscles of the antenna, among which it runs. It seems, then, that the ejaculatory process, by which the secretion of the gland is sometimes forced out of the external orifice, cannot have its origin in any intrinsic action of the duct. It may arise from concerted action of the muscles of the antenna, but since no especially active movement of the antenna has been noted in connection with this function, it is probable that the force producing such ejaculation has its seat in the muscular walls of the vesicle, the duct being passive.

The *external orifice* of the duct is situated at the apex of a truncate conical tubercle, which rises from the general ventral surface of the coxopodite of the second antenna. This tubercle is nearly in the midventral line of the appendage. It does not stand perpendicular to the general surface, but its axis is inclined slightly forward and medianward, so that if the antennæ were at rest in the horizontal position, the axes of the two tubercles prolonged would intersect at a point in the sagittal plane

about 2.5 cm. in front of, and 4 cm. ventral to, the bases of the first antennæ. The posterior and median sides of the tubercle are more elevated from the general surface than are the anterior and lateral sides. The truncated face of the tubercle is a circle with a diameter of about 1 mm., and is covered with a membrane which has concentric markings. The actual orifice is situated in the centre of this membranous area, and is less than 0.1 mm. in diameter. The opening into the tubercle from the main cavity of the appendage, as may be seen by inspecting the inside of a moulted shell, is semicircular, the anterior edge being straight; from this edge a toothed process of the shell, serving for the attachment of muscles, projects backward.

The *blood supply* of the antennal gland is mainly from two sources: (*a*) the antennal artery (arteria lateralis of Gerstaecker); (*b*) the sternal artery. The antennal artery arises directly from the heart, and passes obliquely forward and downward close to the dorsal body wall. In that part of its course where it runs near the vesicle, it gives off several small branches, which are distributed to the lateral wall of this organ. This artery sends branches into the gland proper at three regions: (1.) As the artery passes ventrad along the anterior face of the antennal flexor muscle, a large branch is given off, which goes on the lateral and ventral side of this muscle (i. e. outside of the muscle), and enters the lateral region of the posterior lobe of the gland on its ventral face, to which region it is distributed. (2.) As the antennal artery passes ventral to the lateral anterior lobe of the gland it sends several small branches into the ventral face of this lobe; and one large branch to the bottom of the hilus, whence it passes dorsad to be distributed to the endsac (Plate 1, Fig. 1, *art. sac.*). This branch Marchal ('92, p. 162) terms the "artère sacculaire." (3.) From the dorsal branch of the antennal artery a small branch is given off, which runs ventrad and mediad to enter the median anterior lobe of the gland. It enters on the ventral face close to the anterior edge. The antennal artery after giving off the artery to the endsac passes into the second antenna. In the region of the coxo-basal joint of this appendage two small branches are given off to the walls of the duct and to the tissues at the base of the tubercle.

The second source of blood supply is the sternal artery. A branch from this artery comes to the median surface of the posterior lobe, where it divides. The larger of the resulting branches enters the extreme posterior region of the lobe on the ventral face close to the edge; the smaller passes forward along the edge of the gland and enters the ven-

tral face of the median anterior lobe. Other smaller vessels enter the ventral face of the gland at several points, coming from the trunks supplying the tissues upon which the gland rests.

To summarize, the lateral part of the labyrinth, together with all the endsac, is supplied by the antennal artery, while the median part — except the tip of the median anterior lobe — is supplied from the sternal artery.

The *nerve supply* of the gland is from the nerve trunk running to the second antenna. Immediately after this trunk emerges from the supra-œsophageal ganglion, it gives off a branch which passes outward and backward to enter the median anterior lobe of the gland. The point of entrance is on the ventral face near the median edge. The nerve trunk to the second antenna also sends several small branches into the edge of the median anterior lobe; these arise at points more distal than that at which the main branch arises. I have not found any nerve branches entering at the hilus, nor in the posterior region in a position corresponding to the blood supply from the sternal artery.

B. Finer Anatomy.

The gland proper consists of two distinct parts. The relation of the two parts — the endsac (*sac. trm.*) and the labyrinth (*lby.*) — is seen in Figure 2 (Plate 1), which is a transverse section of the gland proper about half a millimetre posterior to the bottom of the hilus. The endsac is dorsal to and covers the whole of the labyrinth except its extreme edges. Its average dorso-ventral depth at this region is about one fourth that of the labyrinth. This ratio is greater than the average for the whole gland.

The *endsac* shows a considerable regularity in the arrangement of its cavities. At the bottom of the hilus there is a single large chamber of irregular shape, from which outpocketings radiate. These are separated from one another by folds of the wall of the endsac, which form complete septa. These outpocketings branch dichotomously many times, and thus produce a system of compartments which become smaller and smaller until the edge of the endsac is reached. In these compartments there are secondary foldings of the wall forming incomplete septa, which are more frequent on the ventral than on the dorsal wall of the compartments. In Figure 2 (Plate 1) the compartments near the edge of the endsac are cut crosswise; those near the centre, lengthwise or diagonally.

The dorsal face of the endsac has attached to it the closely adhering ventral wall (*par. vs.*, Figure 2, Plate 1) of the overlying vesicle. This

consists, however, of only the glandular epithelium of the vesicle wall, the muscular layer — to be described later (p. 167) — being in this region absent. Between the basement membrane of the epithelium of the vesicle and the wall of the endsac there is a rich plexus of blood-vessels, — branches of the sacculary artery, — some of which are shown cut across in Figure 2 (*vas. sug.*).

The septa of the endsac, both complete and incomplete, consist each of a fold of the epithelial lining of the sac (Plate 1, Figure 3, *cl. sac. trm.*), embracing a sheet of connective tissue (*tis. con't.*) between its two layers. This connective tissue is highly vascular, being principally formed of the walls of blood vessels and blood lacunæ. The glandular epithelium of the endsac forms of course a continuous layer, lining all compartments and investing all septa. It is everywhere a single layer thick, and its cells have certain distinctive characters. In shape they vary both according to location and to condition of activity. They are most elongated on the dorsal wall (Figure 4) and at the place of junction with the cells of the labyrinth; most rotund on the ventral floor and on the septa which rise from it (Figure 3, *cl. sac. trm.*).

The difference of shape due to the state of activity is more striking. Cells lying side by side (Figure 4) vary much in this respect. Among shorter cells of nearly uniform diameter there are many elongated cells, the free ends of which are expanded into globules often in diameter two or three times that of the basal portion of the cell. The contents of these swollen ends are less dense and stain less deeply than do the basal portions of the cells. The globules become detached and pass to the exterior, the lumen of the endsac being more or less filled with various sized globules of this nature (Figure 4, *glb.*). Each, when detached, — if free from mechanical pressure, — is spherical and contains a granular clot nearly filling it. This clot is not homogenous, but made up of granules of various sizes and degrees of opacity. Various progressive stages in the constricting off of the globules from the cell are seen, so that there can be no doubt that they arise primarily from the cells. These globules seem to be composed of a mass of secretion products enclosed in a capsule, which is part of the cell wall. They are not detached cells, for they never contain a nucleus, nor, so far as I have seen, any chromatin particles. Further, there are no evidences of nuclear division in the glandular epithelium, as would probably be the case if these globules were degenerate cells; for if some cells became degenerate and passed off, division would be necessary in the normal cells to make up the loss.

These glandular cells are crowded with a mass of spherical vacuoles, which are smaller in the attached end of the cell than in its free end (Figure 4), and sometimes are not found at all in its basal part (Figure 5). The cytoplasm also contains granules of various sizes and shapes, which differ widely in refractive powers. These are found throughout the cell, often in compact opaque masses. Such masses are also common in the globules (Figure 4).

In sections prepared by certain methods, namely, in material fixed in saturated aqueous-corrosive sublimate, or in vom Rath's platinic-aceto-osmic mixture, acicular crystals are found in many cells, though not in all. Such crystals are not found in material treated with Perenyi's fluid or with Flemming's weaker mixture, nor in any sections which have been decolorized with alcohol acidulated with 1% HCl. These crystals are all fine and needle-like, but differ in length (Fig. 5, *cry.*, and Fig. 6). It is noticeable that they are confined to the cells of the end-sac, none being found either in the lumen of the endsac or in the cells or lumina of labyrinth or vesicle.

Szigethy ('85, p. 109) found in the gland of Astacus crystals which he regarded as uric acid. I have not seen his figures in the original paper, and so cannot compare them with those I have described. In the present case, I think it probable that the crystals are artifacts; at any rate, more detailed investigation is necessary before they can be considered of physiological importance. I have simply made a record of the condition noticed.

The nuclei of the cells of the endsac are oval and usually situated in the basal half of the cell; but in some cases they are crowded toward the free end (Figure 4). The cells rest upon a membrane (*mb. ba.*, Figs. 4 and 5) of appreciable thickness, but without nuclei.

There is *but one region of communication between endsac and labyrinth.* This is in the main axis of the gland immediately posterior to the bottom of the hilus. The section from which Figure 2 is drawn passes through this region. The communication is between the central lumen of the endsac and the lateral anterior lobe of the labyrinth. The median anterior lobe has no direct communication with the endsac, but it will be remembered that it is from the median anterior lobe that the white lobe — which is the direct passage to the exterior — arises (p. 159). Therefore the products of the endsac must pass through the lateral-anterior, the median-anterior, and the white lobes in the order named before reaching the duct to the exterior. The communication between the endsac and the lateral-anterior lobe is about 0.4 millimetre in diameter, and

runs obliquely ventrad and laterad. It is short, and soon branches to become continuous with the lumina of the various labyrinth tubules.

At the border of this short channel, as indicated at α in Figure 2, there is a sudden transition from the cells of the endsac to those of the labyrinth, there being no cells of intermediate character. The boundary of this opening is the only place where the cells of endsac and labyrinth come into contact. At all other places where endsac and labyrinth come together, the basement membranes of the two are face to face.

The *labyrinth* is not a single pocket with evaginations like the endsac, but is composed of a system of branching tubules; these are not, however, simply coiled tubules, for they undergo anastomosis at frequent intervals, and it is therefore impossible to trace any single tubule for a great distance. Moreover, the tubules are not of uniform calibre, but have numerous outpocketings, which still further complicate the appearances presented in sections. This communicating system of lumina is so complicated that it is impossible to say that it presents a definite plan. It is certain, however, that it does not represent one or a few coiled tubules, as has been described for Astacus. The whole system of labyrinthine passages is lined by a continuous layer of epithelium. The basement membrane (Figs. 7 and 8, *mb. ba.*) of this epithelium is somewhat thicker than that of the epithelium of the endsac. The spaces between the epithelial lining of adjacent tubules is occupied by a vascular connective tissue (Fig. 7, *tis. con't.*), like that described in the septa of the endsac (p. 163). The blood-vessels and lacunæ found in this tissue contain blood corpuscles (Fig. 8, *vas. sng.*).

The epithelial cells in different regions of the labyrinth do not show differences of form, but they are very unlike those of the endsac. The cytoplasm is denser and stains more deeply, and the vacuolated condition so characteristic of the cells of the endsac is not seen. Instead, the cytoplasm contains large numbers of granules of very uniform size and power of refraction, and exhibits a striation which is perpendicular to the basement membrane. The oval nuclei lie near the middle of the cell, and are somewhat larger than those of the epithelium of the endsac. But the distinguishing feature of the epithelium of the labyrinth is its free border. This differs in different glands, and in different parts of the same gland; the extreme conditions may, indeed, be seen even in neighboring regions of the same tubule. In Figures 7 and 8 are shown the extremes of the conditions seen. The free border of the cells shown in Figure 8 appears striate, and there is formed a layer which, though it is not a true cuticula, resembles one. The parallel striæ represent partitions

between adjacent vacuoles which form a single superficial layer and are by pressure elongated in the long axis of the cell. In Figure 7 some cells exhibit large globular protrusions from their free ends. The cytoplasm of these protrusions is less dense than that at the base of the cell, and they bear some resemblance to the globules found on the cells of the endsac. Other cells in Figure 7 show two or three small globules, and still other cells might have been shown having a larger number of proportionally smaller globules. I believe that the large globules arise from cells in the condition seen in Figure 8, by the progressive fusion of neighboring small vacuoles at the free ends of the cells, accompanied by an expansion and protrusion of the wall of the free end of the cell. As far as my observations go, I can agree with the main points of Marchal's ('92, pp. 228–230) description of this process, except that I do not find secondary smaller globules *within* the larger ones.

The large terminal globules evidently become detached from the cells, since great numbers of such globules (Fig. 7, *glb.*) of various sizes appear free in the lumina of the tubules. They resemble the globules from the endsac in being spherical, but their contents are much more homogeneous, the granules being nearly alike in size and refractive properties. This production of globular bodies with granular contents characterizes the cells of both endsac and labyrinth, but the manner of their formation differs somewhat in the two cases. There is, then, a typical true secretion by these cells, not merely a filtration of substance through them.

The *vesicle* has a wall without folds or protuberances. It is composed of three layers; the inner one epithelial (Plate 1, Figs. 10 and 11, *la. e'th.*), the outer one muscular (*la. mu.*), and between these a third layer containing a system of blood vessels (Figure 11).

The epithelium is throughout one layer deep. The cells in surface view (Fig. 9) are seen to be irregularly polygonal. They are often elongated parallel to the axis of an underlying blood vessel. The resemblance to the cells of the labyrinth is close, as is to be expected from their similarity of origin (p. 189). The cytoplasm is granular and striate, but shows more vacuoles (Figs. 9, 10, and 11) than occur in the cells of the labyrinth. The nuclei are oval and situated near the middle of the cell. There is a basement membrane of appreciable thickness, which may be separated from the cells by maceration and teasing. The free border of the epithelium (Figs. 10 and 11) presents the same condition as that seen in Figure 8 from the labyrinth; but I do not find the other extreme condition (Fig. 7), with large globules attached to cells, although some of the earlier intermediate conditions between these two extremes are found.

The muscular layer is more variable in thickness than is the epithelial layer. It is composed of long spindle-shaped cells (Fig. 12) of different lengths, containing centrally located nuclei. The cells and nuclei are much flattened in one plane. The edge of the cell is frequently crinkled so as to produce a sinuous outline (Fig. 13). The cell contents are fibrillar, as is well shown when a cell is frayed out in teasing. The fibrillæ are likewise crinkled. There is usually present an axial bundle (Fig. 13, *fas. ax.*) of fibrillæ, which seem to be more closely bound together than are the others; these stain with hæmatoxylin more deeply than the rest of the cell. The thickness of this muscular layer varies. It may be several cells (Fig. 10, *la. mu.*) or a single cell deep (Fig. 11, *la. mu.*), or it may disappear altogether, as it does where the wall of the vesicle is attached to the dorsal face of the endsac (Fig. 2, *par. vs.*). In any given place in the muscular sheath the cells lie parallel with one another, there being no crossing or formation of meshwork.

Between the basement membrane of the epithelium and the muscular sheath there is a layer of connective tissue containing a plexus of blood-vessels. One of these is seen cut crosswise in Figure 11 (*vas. sng.*). These vessels present the ordinary structure seen in the vessels of other parts of the body, and are readily distinguishable from the two other layers of the wall of the vesicle.

The position and external appearance of the tubercle upon which the duct opens has already been described (p. 160). In Figure 14 (Plate 1) is shown a section in the long axis of the appendage through this tubercle. It is seen that the base is much constricted by an infolding of the integument on the anterior side. This is the toothed process referred to on page 161. As the duct passes through this narrow region, it is constricted, but within the tubercle again enlarges. It opens through the middle of the integumental membrane which covers the face of the tubercle. This opening varies in different specimens from 25 to 40 micra in diameter (Figure 14, *of. ex.*). The tegumental membrane is composed of three layers, which, passing from within outward, are, (1) the thin non-calcified layer of the shell; (2) the non-pigmented calcified layer (Figure 14, *la. cx.*); (3) the cuticula or enamel layer. The pigmented calcified layer (Figure 14, *la. cx'.*) stops abruptly at the edge of the truncate face of the tubercle. The three layers of the membrane are traceable into the duct on the anterior side, but not on the posterior. However, even on the anterior side these rapidly thin out and disappear entirely at a short distance from the outer face of the membrane. This continuation of the integument into the anterior floor of the duct forms

a sort of operculum (Figure 14, *op.*). The epidermal cells upon which this rests, and by which of course it has been produced, are much elongated, and their deep ends are attached to a ligament (Figure 14, *lig.*). To this ligament are attached, in turn, other elongated epidermal cells, the basal ends of which are attached to the integument of the "toothed process." I have no evidence that either set of these epidermal cells is contractile, but they are both peculiarly elongated, and if they are contractile we have here a mechanism for opening the external orifice by depressing the operculum. This would allow the escape of the excretory products, they being forced out by contractions of the muscular layer of the wall of the vesicle.

The space between the duct and the wall of the tubercle is filled by the dermis (Figure 14, *drm.*), in which are found tegumental glands (*gl. e'drm.*). At its base are striated muscles (*mu.*) belonging to the antenna, but having no connection with the operculum. On the tubercle, especially around the edge of its truncate face, there are numerous sensory hairs (*set. sns.*).

II. Development.

A. Historical Summary.

The published work on the development of the antennal glands in decapod Crustacea is meagre and lacks both detail and completeness. In most cases, it is only a by-product of researches along some other line, or part of a general consideration of the life history of some species; this may account for the contradictory statements on the subject.

Rathke ('29) in a work much in advance of his time describes (p. 51) for the crayfish embryo, at the time when pigmentation of the optic lobes is well begun, the formation of a slender narrow plate, whose greatest diameter is directed from dorsal-posterior to ventral-anterior. This plate presents a rounded outline dorsally and ventrally; its posterior edge is concave; its anterior convex. The outer surface is in contact with the integument, the inner with the yolk, from which it has its origin "through deposit of plastic material." The duct to the exterior was not made out. These "Speicheldrüsen," while remaining in connection with the yolk, increase in size and become more spherical (p. 60). Just before hatching, there may be seen in these organs small round green spots, the occurrence of which may be assumed to indicate the beginning of secretory activity. When the yolk disappears, they

have a discoidal form, are green in color, and lie against the wall of the stomach (p. 64).

This early account is nearly correct as far as it goes, but it deals with only those parts resulting from the ectodermic invagination; the author failed, moreover, to see the connection with the exterior, which would have shown him the relations of the organ and would have prevented the use of the term "Speicheldrüsen" in relation to them.

The next reference to the embryology of the organ is by Lereboullet ('62), who describes (pp. 760, 761), also in the crayfish, the formation of certain glandular bodies of unknown function at the stage when the stomach grows forward to produce the lateral pouches. These bodies are formed of a simple transparent tube, which in one part of its course is coiled, and is directed toward the base of the corresponding external antenna. The green glandular body also formed by coiling of the same tube is not seen until later.

It is difficult to reconcile this last statement with the preceding, but it seems to indicate that the author believed that there were two glands of different kinds arising in this region, but not simultaneously. The part first referred to is probably the duct to the exterior.

A. Dohrn ('70, pp. 253, 254) describes in the basal segment of the antenna, in the early embryonic stages of Scyllarus arctus, a round cell-mass surrounded by a membrane, and outside this by "einer starken Hypodermisschicht." In Palinurus vulgaris, according to Dohrn, the antennal glands lie in the basal segment of the antenna (p. 261). They are surrounded by the hypodermis, but are free from it. The central part is a cavity in which lie rather close together large free cells, while the wall of the gland is continuous by means of the duct with the ventral and inner wall of the base of the antenna. The free cells within the cavity are probably of the same origin as those of the wall, and ultimately all are incorporated into the wall of the gland. I think it probable that the author erred at this point, for the "free cells" are probably of *different* origin from the others, i. e. of mesodermic origin, and are the Anlage of the endsac.

Reichenbach ('77, p. 191) finds that the green glands arise by invagination of the ectoderm at the stage when the maxillipeds begin to appear, but he gives no figures of them.

Grobben ('80, p. 104) says the antennal glands, as well as the shell glands in Moina, arise from the middle germ layer, but he fails to give arguments, descriptive figures, or authority to warrant this conclusion concerning the *antennal* glands, or even to say in what species it

occurs. I cannot therefore agree with Kingsley ('89, p. 30) in saying that Grobben "showed that the gland belonged to the mesodermal tissues."

Ishikawa ('85, p. 422, Pl. XXVIII. Figs. 68, 90–94) finds in Atyephira, in the base of the second antenna at the time of the appearance of the first pair of maxillipeds, a circular group of abont eight granular ectodermic cells. In these is formed a cup-shaped depression from the exterior, which has a large mouth and grows deeper while the opening narrows. "The cells which are concerned in the formation of it [the antennal gland] are all ectodermic." By the time of hatching, the depression has become a canal having three or four convolutions and filled with granular fluid.

Reichenbach ('86, pp. 97, 98) sees in sections of the embryo of Astacus with well outlined maxillipeds (Stage G) the Anlagen of the glands appearing in the basal segment of the second antennæ as recently formed plugs of ectoderm (Taf. X. Fig. 125 u. 126, *g. D.*). The arrangement of cells shows that a sac is to appear, although no lumen is as yet present. At a slightly older stage (Stage H), with pereiopods marked off, surface views show a crescentic arrangement of cells bordering an involution. The opening of the ectodermic invagination is the permanent mouth of the gland. This invagination grows anteriorly, making a slight turn. The Anlage remains in connection with the ectoderm, and at first does not even approach mesodermic tissue. At later stages (Stage J, with distinct pleopods, and Stage K, with strongly developed eye pigment) flat connective-tissue cells begin to surround and soon envelop the gland (Taf. XIII. Fig. 205).

Thus far, with the exception of the unsupported statement of Grobben ('80), there is a consensus of opinion as to the ectodermal origin of the glands.

With this opinion Kingsley disagrees. He found ('89, p. 29, Plate II. Fig. 49) in Crangon vulgaris a patch of mesoderm stretching into the base of the second antenna as a solid plug at a stage when the eye pigment existed as a crescentic line (Stage G). In a somewhat older embryo (Stage H) "a cavity (Plate II. Fig. 61) appears in this tissue, and the cells lining it take a well marked epithelial character, their boundaries being distinct." At no "stage does the green gland have any connection with any other cavity within the body. . . . The external opening to the gland is not formed until after hatching." By this, I infer, is meant the confluence of the lumina of the endsac and the duct. The mesodermal origin of the antennal gland in Crangon agreed with

that for the shell gland of Phyllopods, as already worked out by Grobben ('79, p. 23), and later confirmed by Lebedinsky ('91, p. 152).

Lebedinsky ('89, p. 197, see also '90, p. 184) has found that the "Segmentalorgan" in the first maxilliped of Eriphia is formed from both ectoderm and mesoderm, and from this he concludes ('92, p. 233) that the antennal glands, the shell glands, and "Segmentalorgane" in Crustacea are serially homologous organs.

Allen ('93, p. 338) says that in a larva of Palæmonetes a few days old the green gland consists of an endsac, which communicates by means of a U-shaped tube with a very short ureter opening at the base of the antenna, and that the distal portion of the tube is slightly enlarged and may be termed a bladder. At hatching, the deeper part of the gland is a mass of cells without lumen, but ureter and external opening are present. Very early in larval life the cells of this deeper portion separate from one another, giving rise to the lumen, and the gland probably becomes functional at that time. The development of the organ in the larva consists chiefly in the enlargement of the bladder, which, arising at the elbow of the U-shaped tube, grows mediad and dorsad to a great extent. The shell gland, opening at the base of the second maxilla, is the functional kidney during embryonic life, but it is not found in young adults.

In his later paper Allen ('93[a]) gives figures (Plate XXXVI. Figs. 1 and 2) showing that in the young larva the endsac differs histologically from the tubule and bladder, the two last being lined with an epithelium composed of cells which are striated and capped with a cuticular structure. The plexus of tubules between the endsac and the bladder arises from the original tubule in that region "obviously . . . by the splitting up of the single tubule" (p. 406).

Boutchinsky ('95, pp. 78, 79, Tab. II. Fig. 51) found that in Iphinoë the ectodermal part of the gland arises as a true invagination from the exterior, with a lumen at all times connected with the outside world. The deep end of this invagination is surrounded by a mass of mesodermic cells. In Gebia littoralis, at a stage (Tab. IV. Fig. 85) when the telson has grown forward so that it is even with the mandibles, he saw (pp. 169, 170) the first appearance of the antennal gland as a compact ball of mesodermic cells (Tab. VI. Fig. 143) with distinct walls and large granular nuclei. The ectodermic invagination occurs at a later stage (Fig. 153), a lumen meanwhile having formed in the mesodermic part of the gland. The mesodermic part soon elongates and becomes horseshoe-shaped (Fig. 154). He reaches two general conclusions in

regard to the embryology of the gland: — (1) that it is formed from an inner mesodermic and an outer ectodermic part; (2) that the formation occurs at a time when the mesoderm shows no regularity in the distribution of its cells, these being scattered without visible order.

I have published (Waite, '98) a short abstract of some of the chief points in the development more fully described hereafter.

B. Development in the Embryo.

In the following account of the development of the antennal glands in Homarus, it should be stated at the outset that it is very difficult to fix definitely the time intervals between successive stages in the embryonic development. The determination of age in hours or even in days is not of much importance. Since the rate of development is known to depend largely upon temperature, the conditions of embryos of the same age must vary with every station, with eggs extruded at different seasons of the year, and also with eggs extruded at the same date in a given locality in different years. Since, then, rate of development is dependent upon many variables, the known intervals between the killing of different embryos in the same series are of only relative value, not being accurately comparable with the intervals of any other series. Further, even in the same series, in which apparently the same conditions have prevailed, there is individual variation.

Notwithstanding these sources of uncertainty, I shall state the approximate age at which the several stages appear, this approximation being arrived at by comparing the several series of my material with one another and with the published figures of Bumpus ('91) and Herrick ('95). I shall give in each case the date of killing, which will indicate the seasonal influences to which the development was subjected.

The earliest embryo which it is necessary to consider in dealing with the development of the antennal gland was killed on August 29, and had reached the late nauplius stage (Plate **2**, Fig. 15). This embryo has the cephalic lobes well marked off, and the first (*at. 1*) and second antennæ (*at. 2*) and the mandibles (*md.*) well defined. The second antennæ are biramous and reach nearly to the posterior limit of the pleonic flexure. Of the post-oral appendages, the buds of the first maxillæ have just begun to appear.[1]

[1] This stage is a little further developed than that figured by Herrick ('95) in Cut 32, Plate I., which he estimates by comparison with embryos raised from seg-

In an embryo of this age I find in sections that the basal region of the second antenna is completely filled with cells having certain distinctive characters (Plate **2**, Fig. 18, *ms'drm.*), which serve to differentiate them from the outer wall (*ec'drm.*) of the appendage. These axial cells are oval or slightly elongate, with rounded outlines, and less uniform in size and less densely granular than the rectangular ectoderm cells (*ec'drm.*), which form the outer wall of the appendage. The nuclei of the axial cells are more oval than the nearly spherical nuclei of the ectoderm cells. In some sections it is to be seen that this plug of axial cells is connected with similar plugs of cells in the base of the first antenna and of the mandible by means of a single layer of cells immediately beneath the ectoderm of this region.

According to recent works on the formation of the germ layers and of the appendages in Decapods, — I refer especially to Reichenbach ('86, p. 24, Taf. IX. Fig. 84, and Taf. X. Fig. 121), Bumpus ('91, p. 245, Plate XVIII. Fig. 6), and Herrick ('95, p. 209), — the cells which form the axis of the appendage are clearly mesoderm, in the generally accepted meaning of the word.

In embryos one day older (Plate **2**, Fig. 16), in which the first maxillæ (*mx. 1*) are marked off, a small number of these axial mesodermic cells in the base of the second antennæ have become differentiated from the rest. *They are destined to become the deeper part of the antennal gland.* These cells are nearly on a level with the general surface of the body. They are larger than the remaining mesodermal cells, and have the cytoplasm less densely granular, especially toward the centre of the cell, and their nuclei also are larger and stain much more deeply with hæmatoxylin. The nuclei are likely to be eccentric, and in many cases they are surrounded by a clear zone of non-granular or very sparsely granular cytoplasm (Plate **2**, Figs. 19, 20, 21, 22, *sac. trm.*).

I believe that there is at first only one of these differentiated cells in each antenna, although I have not found this condition in any of my sections. My reason for thinking so is that in several cases in which only two nuclei are present I have found these close together, and with their adjacent sides somewhat flattened (Plate **2**, Fig 19, *sac. trm.*), as if recently divided. I have not found, however, any mitotic phenomena which might serve as proof of a recent division. On the other hand, it

mentation stages (see his Tables 17 and 18, p. 56) as 14 to 16 days old. With this as a basis, the embryo of my Figure 15 would probably be between 15 and 17 days old. It falls between the Stages N and O of Bumpus ('91, p. 248, Plate XIV.), and is a little further developed than Reichenbach's ('86, p. 47, Taf. III. Fig. 10) Stage G of Astacus.

is possible that there are two cells differentiated at the same time. Sections preceding and following the one seen in Figure 19 show that only two of these differentiated cells are present in this appendage at this stage. In three other specimens also I have found only two such cells, so that there is certainly a two-cell stage in the development of this organ. These nuclei divide, but divisions are not necessarily simultaneous, for I have found cases in which there are three, four (Fig. 21), five, six, seven, eight, nine, and twelve nuclei present. In a few cases mitotic phenomena are found in some of the nuclei, but in most cases, even where an odd number of nuclei is present, there are no mitotic figures. These nuclei are distinctly different from those of the ectoderm and of the surrounding mesoderm in the appendage. There can be no doubt about these differentiated cells being of mesodermic origin. In another particular this region differs from the surrounding mesoderm; although the nuclei increase in number, there is not a corresponding increase of distinctly separated cells. There are partial cell walls (Plate **2**, Figs. 19, 21, *par. cl.*), but the cell areas are not fully or sharply defined by cell membranes. This condition is found in individuals which have been treated by one or other of several different methods of killing, hardening, and staining; it does not appear therefore to be due to the influence of particular reagents.[1] The result is a partial syncytium, or cell complex, in which the nuclei are irregularly distributed, but often show a tendency to take a peripheral position (Plate **2**, Figs. 23, 24).

Bergh ('88, p. 228) has found a parallel condition in the nephridia of Criodrilus. My results do not, however, agree with the condition found by Boutchinsky ('95, p. 169, Tab. VI. Fig. 143) in Gebia, in which, as he says, the cells at a similar stage are separated by distinct walls.

In Homarus this syncytial mass has an even oval outline, the longer axis of the oval lying nearly in the transverse plane of the body, but directed obliquely laterad and ventrad at an angle of 20° or 30° with the frontal plane. The evenly rounded outline of the mass is preserved even where it is separated from the mesenchyme by only a thin layer of mesodermic cells. This oval syncytium with its contained nuclei *becomes the endsac of the antennal gland,* and in the further description will be so designated.

[1] It should, however, be stated that my material had been in alcohol for some time, which may have caused disintegration of cell walls in this particular region. The cell walls of neighboring regions, and of corresponding positions in other appendages, are however firm and distinct.

In an embryo about 22 to 24 days old (September 5th),[1] represented by Figure 17 (Plate **2**), the spheroidal form of the endsac is well marked (Plate **2**, Fig. 23, *sac. trm.*). At this stage, however, when there are twelve nuclei, it is not visible from the ventral surface of the appendage, nor is there any circular or crescentic arrangement of nuclei and cells at the base of the appendage, such as several authors figure as representing the first trace of the antennal gland. Such an appearance accompanies the ectodermic invagination, of which there is as yet no trace, either in surface view or in sections.

In an embryo from the same brood two days older (September 5th to 7th) there appears in sections a definite vacuole (Plate **2**, Fig. 24, *vac.*) situated centrally in the endsac, which here has only nine nuclei. This vacuole has a smooth distinct outline, which is evidence that it is not an artifact. It is clearly not intercellular, for there are no cell membranes near it. From this stage on there are seen in the endsac of each individual a few well marked vacuoles of various sizes (Plate **2**, Fig. 22, Plate **3**, Fig. 26).

It seems probable that the lumen of the endsac originates by the confluence of the vacuoles of the syncytium. I can therefore agree with Kingsley ('89, p. 29) that the lumen of the endsac is neither an enclosure of part of the body cavity nor in any way connected with it. A process parallel to this is described by Bergh ('88, p. 228) in the formation of the nephridia of Criodrilus.

It is interesting to notice that the formation of the lumen of the endsac begins before there is any trace of the ectodermic invagination, and only eight days (August 30th to September 7th) after the earliest differentiation of the endsac is seen, whereas in Palæmonetes (Allen, '93, p. 338) the lumen of the endsac does not appear until early in larval life and is there intercellular.

The next stage to be noticed is from another series. It is slightly older than that figured by Herrick ('95) in Cut 34, Plate I., and between Stages O and P of Bumpus ('91, Pl. XIV.). The tip of the telson has grown cephalad until it is somewhat in advance of the base of the second antenna; there is no pigment in the eye. There have elapsed since fertilization, to judge from a comparison with Herrick's figures, 23 to 25 days, but the actual interval is greater, — probably 28 to 30 days, — as

[1] This embryo was from the same brood as that from which Figure 16 was drawn, showing the earliest recognized condition of the endsac. Since the younger was killed on August 30th, and the older on September 5th, there is an interval of six days between the two.

these embryos were killed in October. In most individuals of this age there is no sign of ectodermic invagination in the region of the endsac. The endsac (Plate 2, Fig. 25, *sac. trm.*) is oval and contains in different individuals from 18 to 26 nuclei, which are often only partially separated by cell walls, these being evident in the peripheral portions of the organ only. The central area of the endsac is a granular mass with one or more large irregular vacuoles, or several small ones, while the nuclei lie peripherally in compartments between the incomplete cell walls. In some cases the cell walls are completed, and thus a definite boundary to the lumen is formed. This is the condition of the endsac when invagination of the ectoderm begins, but in some individuals, even after invagination is well started, the cell walls of the endsac are incomplete and its interior is still a granular mass with several vacuoles (Plate 3, Fig. 26, *sac. trm.*, *vac.*).

In some precocious individuals of this batch of embryos I find evidence of the beginning of the invagination, — a slight depression of ectoderm cells. In Figure 25 (Plate 2) at the median ventral border of the endsac appears a thickening of the ectoderm (*i'vag. ec'drm.*). This represents the beginning of the ectodermic ingrowth.

In sections of the stages succeeding this it is seen that the ectodermic growth extends proximad and dorsad.[1] The axis of this line of growth is not parallel with the antero-posterior axis of the body of the embryo, but in passing proximad trends slightly laterad, so that true parasagittal sections of the embryo cut the invagination obliquely. The ingrowth consists of a plug, or perhaps better, a sheet of cells, the width of the sheet being however small. This sheet grows up around the endsac and lies against its distal and dorsal faces (Plate 3, Figs. 26, 27, *i'vag. ec'drm.*).

From all the evidence afforded by my sections I believe that this sheet of cells arises by a process of growth which is most active at its free (deep) end. The conclusive evidence must rest upon signs of mitosis in numerous series of stages covering the period of this growth. I have not sufficient material at command to settle the point definitely.

However this may be, this ectodermic ingrowth occupies a narrow space between the distal and dorsal faces of the endsac and the wall of

[1] In referring to the embryonic organ I must use designations of direction which seem contradictory. For that face of the second antenna which lies nearest the mandible and is now the dorsal face is ultimately the ventral, and must be so designated. It assumes its normal position at the time of hatching by revolving about its base as a centre through an arc of about 135° in a parasagittal plane.

the appendage. As a result, in a parasagittal section — at this stage a proximo-distal section of the appendage — there is to be seen a single row of cells (Plate 3, Fig. 26, *i'vag. ec'drm.*). Preceding or following sections of the series usually show that there is an accompanying row by the side of this, i. e. the sheet is two cells wide, and one cell deep dorso-ventrally. In a few cases there is evidence in transverse sections that there are three rows through at least part of its course, these being so arranged that the cross section is triangular. These rows are not distinctly separated but interdigitate, so that the dividing line is anything but straight and sharp, as would be the case were this formation an actual invagination. The line — there is no lumen — between these rows may represent a potential lumen derived from the outside world, but I can trace no actual connection. There is, to be sure, in the region of the connection with the ectodermic wall, a slight depression (Plate 3, Fig. 26, *fos.*) in the surface of the ectoderm, but the cellular arrangement does not warrant the conclusion that this is a real invagination. I believe therefore that the *ectodermic ingrowth is a solid plug, and not an invaginated sac.*

As this plug advances along the dorsal (now ventral) face of the endsac and approaches the proximal border, it is subjected to less stress, its proximo-dorsal border being in contact with only the mesenchyme and the fluids of the body cavity. The plug here enlarges; at first there are in this region one or two extra rows of cells (Plate 3, Fig. 26, *i'vag. ec'drm.*), — cross sections often show four rows, — a little later the plug enlarges into a knob-like body in which an intercellular lumen appears (Plate 3, Fig. 27, *i'vag. ec'drm., lu.*). There is no reason to believe that the lumen thus arising is an enclosed part of the body cavity, and there is no evidence to show that it is actually a part of the outside world. I believe that it is a lumen of independent origin, just as is that of the endsac; but in the case of the ectodermic ingrowth it is *inter*-cellular, while in the endsac it begins as an *intra*-cellular space.

This ingrowth of the ectodermic plug and its expansion into a knob containing a lumen is a rapid process. I have found all the stages hitherto noted in the same batch of eggs from a single female, and all killed at the same moment. In September and October this condition is attained in about 30 days, probably in somewhat less time in eggs extruded in the middle of summer.

The condition shown in Figure 27 (Plate 3) progresses distad and ventrad along the ectodermic plug towards the region of its connection with the ectodermic wall of the appendage. The axial line separating

the cell rows is now distinct and even, and quite different from the line separating the two interdigitating rows of earlier stages. This axial line represents a potential lumen, and by separation of the apposed walls the lumen is formed here at later stages. Figure 28 (Plate 3) is a parasagittal section from an embryo about 35 days old. The endsac (*sac. trm.*) has its cell walls well marked and the lumen (*lu.*) is sharply bounded. The ectodermic ingrowth (*i'vag. ec'drm.*) shows in longitudinal section two rows (many rows in all) to a point near the distal end of the endsac. The cells are more columnar than in earlier stages. Some of the ectodermic cells of the wall of the appendage are much elongated. They go to form the ligaments seen in this region in later stages.

Figure 29 (Plate 3) is a transverse section at a little later stage (about 40 days, September 10th to October 20th). Here the lumen of the ectodermic plug (*i'vag. ec'drm.*) is evident and can be traced to the exterior (*of. ex.*). This duct has arisen as the outward extension of an intercellular lumen, first appearing in the region of the deep proximo-dorsal end of the ectodermic plug.

There is thus formed in the embryo of about six weeks an endsac with definite lumen, and an ectodermic ingrowth with lumen, and with a duct opening to the outside world, but these two lumina are not in connection. The duct of the ectodermic ingrowth persists as the permanent duct of the adult organ. However, its lumen is not always visible, especially in the deeper regions, for the growth and resulting pressure at times apparently obliterate it by causing a close apposition of the walls, which, however, do not actually fuse, for at all subsequent stages when a section in the region is torn the lumen is reopened.

There are still two important points to be determined in the embryology of the antennal gland. When, and at what point, does the lumen of the endsac become continuous with that of the ectodermic sac? In a series of embryos taken from a female kept in confinement during the winter (see Herrick, '95, No. (3) 18, Table 18, p. 56) I find that in those killed April 1st (estimated age 273 days) the lumina are separate, but in embryos of the same series killed May 1st (estimated age 303 days) the lumina are continuous. The details of the union of the two lumina I am unable to describe, as I have no intermediate stages.

For some time before this last stage is reached, the lumen of each part is seen in sections, but the two lumina are separated by the closely apposed walls of the two sacs (Plate 3, Fig. 31). The break through the two walls occurs on the dorsal face of the proximal region of the endsac and at the terminal end of the ventral part of the forked proximal end of the

ectodermic sac (cf. Fig. 30, Plate 3). After communication is established, an abrupt transition in the character of the wall is still noticeable at the point of union. This level may be termed the mes-ectal line, since it is the line of junction of the mesodermic with the ectodermic part of the organ. There is a valve-like flap of cells projecting from the wall of the endsac into the orifice connecting the two cavities. This condition is still evident in the first larva (Plate 6, Fig. 51, *vlv.*).

The brood of this female kept in confinement during the winter was hatched 33 days after the killing of the specimens which showed connecting lumina (about 330 days after fertilization), so that when the endsac comes into communication with the exterior the embryo has already passed through ten elevenths of its embryonic period. It is possible that the establishment of the communication between the two sacs occurs in the earlier part of the period embraced between the ages of 273 and 303 days, but even if it does not occur until the latter part of the period, the lumen of the endsac is in direct communication with the exterior at least a month before the end of embryonic life. The only records that I find of the time when this connection is effected in other Crustacea is that made by Allen ('93, p. 338) for Palæmonetes, and by Kingsley ('89, p. 30) for Crangon. Kingsley states that "the external opening to the gland is not formed until after hatching." Allen says that the formation of the lumen of the endsac and its communication with the exterior occur early in larval life. In Homarus, however, the lumen of the endsac communicates with the outside world late in embryonic life. If, as Allen thinks, the establishment of this condition marks the beginning of functional activity, then the antennal gland in the lobster is functional as an embryonic organ. But I cannot agree with Allen. On *a priori* grounds we may expect an excretory organ in the lobster embryo. Excretion takes place in many yolk-bearing embryos, and special embryonic organs having this function occur in many cases, such as the embryonic nephridia of Annelids and pulmonate Gasteropods, and the shell gland in the embryo of many Crustacea. In some decapod Crustacea the shell gland is functional in the embryo, but atrophies soon after hatching. Whether it is present in Homarus I am unable to say; but I believe it probable that excretion in the embryo is performed by some such special organ rather than by the antennal gland, for in the latter I find no direct evidence of excretory activity during embryonic life. The cavity of the gland contains neither a granular clot nor excretory globules, such as are found in the larval and adult stages, nor have the cells of the wall of the ectodermic sac the marked striation which is seen later when glandular

activity certainly exists. It may well be that excretion in the embryo is less abundant than in the predatory larva, because of the less active metabolism and the more perfect food supply of the yolk. The presence or absence of excretion products in the organ during embryonic life might be demonstrated on fresh material by micro-chemical tests, and the question thus definitely settled; but my material is not so preserved as to permit the application of such tests.

After the formation of the ectodermic ingrowth, as shown in Figures 26 and 27 (Plate 3), the further embryonic development consists, then, in four chief phases: — (*a.*) The line representing its lumen extends to the outside world and so establishes a potential duct. This is a rapid process and is completed shortly after the stage shown in Figure 28 (Plate 3). The condition previously noted at the deep end of the ectodermic ingrowth — several rows of cells — progresses and finally reaches the exterior near the point where the ectodermic ingrowth first appeared (Fig. 25). This potential duct is open in many cases (Fig. 29). The opening of the duct is on the anterior side of the appendage, somewhat toward its ventral face. The ectodermic duct and sac extend from this external orifice dorsad and proximad, covering the distal and dorsal faces of the endsac, but not until a later stage does it pass ventral to the endsac, nor ever much beyond its proximal border.

(*b.*) The ectodermic sac becomes extended and complicated throughout embryonic life by the formation of evaginations, which begin to appear as soon as the lumen of the duct has been extended to the external world. These outgrowths are flattened, probably by mechanical pressure. There are three regions at which principally these evaginations occur. One of these is proximal, i. e. at the deep end of the ectodermic ingrowth. Here the outgrowth soon becomes forked, there being a ventral portion nearer the endsac and a proximal portion more nearly in line with the axis of the appendage. Figure 30 (Plate 3) shows these forks, but the proximal one is seen only in cross section. It extends at right angles to the main axis of the appendage and to the plane of the section, approximately parallel to the frontal plane of the embryo. The notch separating these forks never becomes very deep, but it is recognizable from the second month of embryonic life until the late larval stages. On the dorsal face of the ectodermic sac in the region of its sharpest curvature is a second area of evagination. This outgrowth, which is dorsad, does not exist at the stage shown in Figure 30, but it appears slightly later. It has a considerable horizontal extent (Figure 31, Plate 3), being all that part of the ectodermic sac which later lies distal to the duct. The

third region is along the entire length of the ectodermic sac in the dorsomedian area. An idea of the position of this region may be gained from Figure 31 (Plate 3), which is a transverse section. It is there represented by the long dorsal tract of the ectodermic sac, extending mediad near the letters "*sac. trm.*"

The complications in the shape of the ectodermic sac arise from the differential growth in these three regions. The ventral fork (Figure 30) grows ventrad around the proximal face of the endsac, and then distad between it and the wall of the appendage, meanwhile extending laterad and mediad in the frontal plane. It is this extension that finally connects with the endsac. The proximal fork has grown at the same time, but extends more nearly in the axis of the appendage. Meanwhile the dorsal extension has grown considerably dorsad and distad, and the dorsomedian evagination has extended horizontally. As a result of all these growths the parts of the ectodermic sac have come to embrace the endsac on all sides, though not completely, for it is seen in the larval stages that through a part of its extent the endsac lies against the ventral and lateral walls of the appendage.

All these growths and extensions of the ectodermic sac are evaginations, and carry with them part of the original lumen, but they are *in no respect convolutions*. The complicated lumen thus arising is suppressed, as far as actual cavities are concerned, until the latter half of embryonic life. In an embryo of 152 days (July 1st to December 1st) no actual cavity is observable, only the line marking the meeting of apposing walls, but in an embryo of 303 days (July 1st to May 1st) the lumen is an actual cavity at almost all points. In stages intermediate between these the lumen becomes more and more open as age advances. We thus see that the distention of the lumen occurs in large part prior to the junction (see p. 179) of the lumina of the endsac and ectodermic sac.

(*c.*) The endsac does not suffer much complication during its embryonic growth, but it becomes much flattened and compressed between the extensions of the ectodermic sac. The chief growth is laterad and dorsad, and gives rise to the condition found in the first larva, where the endsac lies in large part lateral to the ectodermic sac (Figure 40, Plate 5).

(*d.*) Finally the lumina of endsac and ectodermic sac become continuous (see p. 178), and the organ has then practically reached the larval condition.

C. Development in the Larvæ.

(*a.*) *In the First Larva.*

The stages at which the various phases of the development of the antennal gland in the embryo appear are not precisely fixed by time intervals because of seasonal variation in the rate of development. They may be referred to particular conditions of prominent external organs, such as the pigmentation of the eyes or the position of the tip of the telson, with much greater precision, but even this allows of no close comparisons with parallel stages in other genera or families. The stage at which the embryo hatches, marking the transition from the passive embryonic to the active larval life, is sharply defined in most free-living Crustacea, and it would seem that this is a time at which comparisons may be made most safely. Moreover, the transition to the predatory life of the larva must have a marked influence upon metabolism, and therefore upon the excretory organ and its functions. It has therefore seemed well to consider this stage in some detail.

My material was all killed within twelve hours after hatching, and hence represents the earlier part of the first larval period, the extent of which may be as much as five days (Herrick, '95, p. 172).

1. General Structure.

With the hatching of the embryo a considerable alternation occurs in the topography of the antennal gland, but this is due to mechanical causes rather than to growth. The second antenna, which has been folded back against the embryo so that its distal end is posterior to its proximal end, is released in hatching and swings — approximately in a parasagittal plane — through about 135 degrees, to a position in which the distal end is anterior to the proximal. Thus in the basal region of the appendage the dorsal face is shortened and thereby relieved of the stress which has existed at that place during embryonic life. This removal of pressure from the extensions and evaginations of the ectodermic sac brings about a change in its form, which passes from the flattened constrained shape to a more rounded one. This permits the separation of the apposing walls and the establishment of a large lumen. Likewise the endsac, which has been compressed between the dorsal and ventral portions of the ectodermic sac, is relieved from this pressure and expands as much as the larger space allows.

In Figures 32 to 38 (Plate **4**) are shown the second, third, fifth, sixth, eighth, eleventh, and twelfth sections respectively from a series

of thirteen sections 10 micra thick through the left antennal gland of the first larva. The sections are cut parallel to the long axis of the antenna and perpendicular to the frontal plane of the larva, and are viewed from the lateral face, Figure 32 being the most lateral one shown. The members of the series reproduced do not extend near enough to the median plane to include the opening of the duct to the exterior, but the relations of this duct to the ectodermic sac and to the external orifice may be inferred from Figure 38.

The ectodermic sac — the walls of which are represented in a lighter shade than those of the endsac — is oval in shape, flattened ventrally, and elongated in the axis of the antenna (Figures 32 to 38, *sac. ec'drm.*). The endsac (Figures 33 to 35, *sac. trm.*) lies in a depression of the ventro-lateral face of the ectodermic sac, as is readily to be understood from its position in the sections and in the series as a whole. The three evaginations of the ectodermic sac — proximal, ventral, and dorsal — are still indicated, though less prominently than in the embryo. The passage from the lumen of the endsac to that of the ectodermic sac — partly closed by the valvular flap — is shown in Figure 33.

The endsac (Figures 33, 34, *sac. trm.*) now lies closely applied to the ventral wall of the ectodermic sac, a relation which is changed in later stages (see p. 190). A ventral recess of the ectodermic sac shown in Figures 37 and 38 leads distad and mediad to the duct communicating with the external world. This is better shown in Figure 39, a section of the left gland from another series, but viewed from the *median* face. This being cut in a more favorable plane, at a slight angle with the long axis of the appendage, shows that the ventral recess continues as a duct to the external opening (*of. ex.*) at the summit of a papilla on the ventral wall of the appendage. It is clear from this figure that the duct is connected with the ventral face of the ectodermic sac. The position of the duct marks the original course of the ectodermic ingrowth in the embryo.

The conclusions reached by the examination of such parasagittal sections are supplemented by the examination of transverse sections. Figure 40 (Plate 5) is the anterior face of a transverse section through the first larva in the region of the base of the second antennæ, and shows the general position of the antennal glands, which lie almost wholly within the appendage. Figures 41 to 48 (Plate 5) exhibit the anterior faces of the third, fifth, seventh, ninth, tenth, twelfth, fourteenth, and seventeenth sections of a series through the right gland, Figure 41 being the most anterior. The sections, each 10 micra thick,

are cut perpendicular to the long axis of the antenna. No section plane passes through the external orifice of the duct, but the orifice is near the plane of the section shown in Figure 46. The opening to the exterior, it will be observed, is not at the middle of the ventral face of the appendage, but more toward the median side. The communication between the lumina of the endsac and ectodermic sac does not appear, as it is small and its axis is inclined to the plane of the section (cf. Figure 33), but it lies between Figure 47 and the next following section. Its position is indicated in Figure 47 by *a*. It will be noticed that the endsac lies partly ventral and partly lateral to the ectodermic sac (Figures 44 to 48), and that its free margin extends dorsally, while the ectodermic sac extends ventrally. This is the beginning of a process of growth that is destined to reverse the relative positions of the two parts as found in the embryonic stages, where the endsac was ventral and the ectodermic sac dorsal (cf. Figs. 26, 27, 28, Plate 3).

Finally, Figure 49 (Plate 6) represents the dorsal face of a frontal section of the right gland, — the tenth, counting from the dorsal face of the gland, in a series of eighteen, each 10 micra thick, — in which is seen the lateral position of the endsac and the communication between the endsac and the ectodermic sac at *a*. The passage is situated at the lateral and proximal part of the endsac.

The evidence from sections in the three planes shows that the endsac lies ventral and lateral to the ectodermic sac; that the ectodermic sac is elongated in the axis of the antenna, and gives off the duct to the exterior from its ventral face well over on its median border; and that the gland lies almost wholly within the appendage and not in the cavity of the body proper.

The greatest diameter of the gland measured in each of the three axes gave as a result of the average [1] of several cases the following: —

Maximum proximo-distal axis, 0.3 mm.
" dorso-ventral axis, 0.2 "
" latero-median axis, 0.2 "

The average length of the first larva is 7.8 mm. from tip of rostrum to tip of telson.

[1] The extremes of measurement vary from these mean averages 20% in some cases, which is probably due to slight differences in the age of larvæ, or to individual differences independent of age.

2. Histology.

The histological character of the endsac differs distinctly from that of the ectodermic sac, and the dividing mes-ectal line is sharply marked (Plate 6, Fig. 51, *ln. mes-ec.*). The valve-like flap (*vlv.*) in the orifice between the two sacs lies on the dorsal side, and its cells are connected directly with those of the wall of the endsac, and are of the same nature. In the region where the walls of the two sacs are adjacent, they are not applied to each other continuously, but are separated by spaces (Figure 51, *lac. sng.*) which constitute blood lacunæ. This is proved by the frequent presence in them of blood corpuscles (Figure 59), the structure of which is characteristic. These blood spaces are separated at intervals by partitions or pillars (Figures 49, 51) connecting the basement membranes of the two sacs. In structure these partitions resemble closely the tissue of the endsac. I have not traced the continuity of these blood spaces with the general circulatory system. They are not lined by an endothelial layer, as far as I am able to discover.

The cells of the endsac are all of the same general type, but show two different forms, one (Fig. 52, Plate 6) being more flattened than the other (Fig. 53, Plate 6). The more flattened cells occur in those regions of the wall which are not adjacent to the wall of the ectodermic sac (Fig. 51). All of the cells of the endsac have large nuclei, which lie close against the free wall of the cell and in most cases cause a protrusion of the cell into the lumen (Figs. 51, 53). The nuclei in the flattened cells are more elongated in a direction parallel to the surface of the wall than is the case in the more rotund cells of the other region (cf. Fig. 52 with Fig. 53). Both kinds show a chromatic network. By the methods used it was impossible to demonstrate any definite lateral cell boundaries, but the extent of the cell territories is indicated by the position of the nuclei and by the contour of the free face of the cells. The cytoplasm is finely granular, and in most cases distinctly striated, the rows of granules being perpendicular to the surfacs of the wall of the sac (Fig. 53). This granular striation would seem to indicate a secretory activity. There is, however, no bounding striate cuticula on the inner face of the cells. The wall of the sac presents a basement membrane, which is however much less marked than that of the wall of the ectodermic sac, and appears as a single line even under the amplification of a $\frac{1}{18}$ oil immersion (Zeiss) and ocular 4.

The cells of the ectodermic sac are very different in character from

those of the endsac, and the transition at the mes-ectal line is, as I have said, abrupt, there being no cells of intermediate character (Fig. 51, *ln. mes-ec.*). The cells of this ectodermic part differ considerably in size and shape, although not essentially in structure. Figures 54 to 57 (Plate 6) are all drawn from the same series of sections, and with the same amplification, but from different regions in the wall of the ectodermic sac; they fairly illustrate the variation in the size and the shape of the cells. The cells from the dorso-median wall (Fig. 56) are more columnar, and the nuclei are relatively farther from the basal end of the cell than in other regions. Those from the ventral face (Fig. 55) are much smaller and show fewer striations. The cytoplasm in all these regions is granular, more densely so than in the endsac, and in all but the ventral wall (Fig. 55) the granules are arranged in rows perpendicular to the basement membrane and separated by clearer areas of periplasm. This striation is found both in the basal and free ends of the cell. In some cases the clear area between rows of granules is expanded to form large vacuoles (Fig. 56, *vac.*). The nuclei are bounded by a definite nuclear membrane and have a coarse chromatic network, which stains deeply in hæmatoxylin. Where the strands of this network cross, there are formed chromatic masses of greater density; these are usually triangular in section (Figs. 54, 57, and 58). Nucleoli are found in some cells (Fig. 55).

Mitotic phenomena, though not abundant in these cells, are now and then met with. One such cell in process of division is shown in Figure 57. The plane of division is in all cases perpendicular to the basement membrane, so that the tissue remains only one layer thick. The rarity of these indications of cell division, in spite of the considerable increase in the size of the gland in the beginning of the second larval stage, is perhaps an indication that the cell increase in the organ is more rapid in the latter part of the first larval period than in the earlier part, at which time my material was killed.

The basement membrane is seen in all cases. Under high powers it has an appreciable thickness (Fig. 58, Plate 6, *mb. ba.*). I have seen no trace of nuclei in it to indicate an endothelial nature.

All the cells of the ectodermic sac agree in having a vertically striate cuticula on the free face (Figs. 54–58, *cta.*). Under high magnification this cuticula is seen to be made up of bundles of rods or strings of granules grouped into the shape of an hour-glass, the clear spaces between these bundles having in section a lenticular shape (Fig. 58, *cta.*). This cuticula agrees in a measure with that in the walls of the labyrinth of

the adult gland, but in this larval stage there are no globular prominences extending out from it into the lumen.

The histological structure of the duct is shown in Figure 60 (Plate 6), which is from a longitudinal section of the appendage. Its wall is directly continuous with the body wall. The dorsal lip of the orifice (*of. ex.*) shows no abrupt transition from the wall of the duct to that of the body. On the ventral lip the limit is somewhat more precise owing to the sharp folding, but it is difficult to say to which wall the apical cells of the ventral lip belong. The cells of the dorsal wall show differences in size and shape among themselves, and a considerable difference from the cells of the ventral wall; these being smaller and imbricated. The cells of the duct do not show the granular striation characteristic of the cells of the ectodermic sac, nor is there a striate cuticula on the face of these cells. These two characters serve to distinguish the cells of the duct from those of the ectodermic sac, and lead to the inference that the former have no secretory function. However, as one follows the wall of the ectodermic sac as it merges into the wall of the duct, these two characters gradually disappear, and it is impossible to say that there is any definite line where ectodermic sac ends and duct begins, — a condition to be expected from the fact of their having had a common origin.

The cuticular layer of the external shell bends into the duct (Fig. 60), but soon thins out and disappears. It extends for a greater distance along the dorsal than along the ventral wall.

The lumen of the ectodermic sac contains a granular coagulum, which resembles in staining properties that seen in various parts of the adult gland, but there is no trace of the globular structures there seen (p. 166). This coagulum is in all probability the secretion of the walls of the ectodermic sac, indicating that the functional activity of the gland has already begun; but that function must differ in some respects from the function in the adult, as indicated by the absence of the globules. This coagulum is not seen in the lumen of the endsac. The identity of the substance found here with that found in the adult gland can only be established by micro-chemical tests, and for that my material is not suited.

It seems probable that the function, although possibly somewhat different from that of the adult, is of essentially the same nature, i. e. excretory. This would be expected both upon *a priori* grounds and from the structure of the cells as described. At this stage metabolism, consequent upon the activity of the larva, is constant, and as all the structural conditions appear favorable, it seems as if this organ, which

later is certainly the chief, if not the only, excretory organ of the body, must be functional and functional in the way of its later activities. I therefore believe that the antennal gland in the first larva is a functional excretory organ.

(*b.*) *In Older Larvæ.*

The antennal gland in the *second larva* shows no marked difference from that in the first, either in shape or in histology. There is, however, as compared with the first larva (see page 184), a difference in size, which again taking averages, is now :

Maximum proximo-distal axis, 0.3 mm.
" dorso-ventral axis, 0.3 "
" latero-median axis, 0.25 "

If we assume, in view of the simplicity of the organ at these stages, that its secreting surface is about equal to the *surface* of a parallelopipedon having the dimensions of the whole organ, we find that the area of the secreting surface in the second larva is 40–50 per cent greater than in the first. The average length of the second larva (see Herrick '95, Table 34) is only about 13 per cent more than that of the first. If we compare the cubes of these dimensions we get an increase of something over 40 per cent in the bulk of the larva; consequently the increase of secreting surface in the antennal gland simply about keeps pace with the increase in bulk of the larva.

In the *third larva* we get the beginning of that process of complication by which the antennal gland of the adult animal is developed from the relatively simple organ of the time of hatching. Between the conditions in the first and second larvæ there is only the difference of size. Between the conditions of the second and third larvæ there is a difference due to increase in size, by reason of which the gland comes to lie largely in the body instead of in the appendage only, and in addition a difference resulting from the development of long slender evaginations on the dorsal and median faces of the ectodermic sac. These evaginations have only a very small lumen, but the lumen is always in connection with the main cavity of the ectodermic sac, as is shown in Figure 50 (Plate 6, *evag.*). By such evaginations the secreting surface is greatly increased without a correspondingly large demand for space in the body cavity.

In the third larva there also begin to appear at the free ends of the cells composing the wall of the ectodermic sac the peculiar globular vesicles which form so marked a feature of the adult gland. They are less well marked and less abundant here than in the older larvæ.

In this stage, the vessels and blood spaces (*lac. sng.*) which lie between the endsac and the ectodermic sac are for the first time seen to be lined with endothelial cells. This vascular tissue has arisen from the cells which are between the endsac and the ectodermic invagination, and which are seen at later embryonic stages (Plate 3, Fig. 30) as attenuated cells forming a sort of sheath around the endsac.

In the *fourth larva*, the evaginations from the ectodermic sac have further increased in number; those which occupy the positions of the ones found in the third larva have elongated, and in some cases the free end is folded back upon itself. On the dorsal face of the ectodermic sac, well forward, there is an evagination which is larger and more rounded than the others. It contains a large cavity which is connected with the main cavity of the ectodermic sac by a narrow duct. This evagination is directed backward and dorsad; *it is the first differentiation of the vesicle of the adult gland.*

The globular vesicles on the free ends of the cells in the wall of the ectodermic sac are more marked, and appear very much like the condition in the adult.

My material of the older larval stages is meagre and not in good histological condition. It is difficult, in the absence of data as to the number of moults passed, to determine to what larval stage a given specimen belongs. The sole criterion available is therefore that of length.

In *larvæ 14 millimetres long*, the dorsal rounded evagination representing the vesicle has increased much in size, extending caudad and dorsad, until its walls are in contact with those of the masticatory stomach. The narrow slender evaginations have increased in number, especially in the ventral part of the organ, and are so entangled that it is futile to attempt to follow details.

Larvæ 18 millimetres long are the oldest that I have. According to Herrick ('95, Table 34), this length indicates the seventh larval stage, and life at the bottom of the sea. In these larvæ the vesicle has enlarged still more, its walls becoming thinner, until it covers dorsally nearly all the organ. The complication of the slender evaginations is now still greater, and anastomoses between adjacent evaginations are established at many points by a breaking through of their walls. This condition is found sparingly in the fourth larva, but in this (seventh?) stage it is quite common.

I have no material of adolescent stages in which to follow this process, but there seems every reason to believe that it is by these evaginations, with subsequent anastomoses, that the complication of tubules in the

labyrinth of the adult gland is attained, and not by any system of coiling of one or a few tubules.

I have already (p. 180) called attention to the fact that in the embryo the endsac is ventral to the ectodermic sac, whereas in the adult organ the endsac is dorsal to the mass of ectodermic tubules (labyrinth) which are developed from the ectodermic sac. How is this change of position brought about? In the first larva — as is seen in Figures 33–35 (Plate 4) and Figures 40, 44–48 (Plate 5) — the endsac lies chiefly ventral to the ectodermic sac, but also extends in part dorsad up along the lateral face of the latter. Sections of later larvæ show that the chief region of growth in the endsac is in this dorso-lateral projection, while in the ectodermic sac the chief area of growth and complication by evagination (see p. 189) is in the ventral region, by means of which it extends laterad ventral to the endsac and between it and the ventral wall of the appendage. There is, then, by this differential growth a rotation of the centre of mass of each of these parts of the organ. This rotation is around the antero-posterior axis of the gland, and, if one imagine himself viewing this from an anterior point, this rotation is clockwise in the right gland, and the reverse in the left. There is no actual twisting, but only a change in position of the centre of mass of endsac and of ectodermic sac. In the larva 18 millimetres long this process has progressed so far that the plane which separates endsac from ectodermic sac is approximately parasagittal in position. Since the antero-posterior extent of the endsac is much less than that of the ectodermic sac, the former can grow dorsad and then mediad without disturbing the stalk which connects the evaginated vesicle with the ectodermic sac, because the endsac lies entirely posterior to this stalk. This process also makes it easy to understand how it comes about that the endsac in the adult gland empties into the lateral anterior lobe of the labyrinth (see p. 164), but has no direct connection with the median anterior lobe.

I have not at my command the material to enable me to follow this process of differential growth in the adolescent stages; but as far as I have been able to examine the larvæ, the conditions found agree with the process which I have mentioned, and I believe that it is in this way that the relative position of ectodermic sac and endsac as found in the embryo comes to be reversed in the adult, where the endsac is dorsal to the ectodermic sac or labyrinth.

III. Theoretical Considerations.

A. Homology of the Antennal Glands with the Nephridia of Annelids.

Leydig ('60, p. 28) was the first to suggest that the antennal glands of Crustacea and the nephridia of Annelids performed similar duties. He, however, ascribed to both a respiratory instead of an excretory function. Since that time there has been much discussion in regard to the functional likeness of these organs in the two groups of animals. Naturally, this consideration has led to the discussion of their possibly being homologous. This discussion was inaugurated by Kowalevsky ('71). Upon grounds of analogy, and from a comparison of the structure of the adult organs, it came to be pretty generally accepted that this homology was valid. Confidence in this view was, however, shaken by the publication of Reichenbach's ('77) description of the ectodermic derivation of the antennal gland in Astacus. Kowalevsky ('71) had given proof of the mesodermic origin of the nephridia in Euaxes (p. 19) and in Lumbricus (p. 25) and had formulated the germ-layer theory, holding "that the homologies of the germ layers in different types afford a scientific basis for comparative anatomy and embryology, and must be recognized as the starting point for the proper understanding of the relationships of the types" (p. 60). So great was the influence of the germ-layer theory that the results of Reichenbach in deriving the antennal gland of Astacus entirely from the ectoderm served, for the time being, completely to check its comparison with the mesodermic nephridia of Annelids.

Grobben ('79) showed, however, that the shell gland of Moina was in part mesodermic, and thus partially restored the grounds for homologizing it with the nephridia of Annelids; but Ishikawa's ('85) evidence that the antennal gland in Atyephira is derived solely from the ectoderm, and the conclusions reached soon after in Reichenbach's ('86) memoir, — in which the ectodermic origin of the antennal gland in Astacus was described and figured in detail, — not only reaffirmed the obstacles to establishing a homology between the antennal glands and the nephridia of Annelids, but also placed in different categories the shell glands of Entomostraca and the antennal glands of Malacostraca.

The contrary conclusions reached by Kingsley ('89) in his description of the development of the antennal gland in Crangon threw some doubt upon the accuracy of the observations and interpretations of

Reichenbach, and served to restore tentatively confidence in the homology of the antennal glands of Malacostraca with the nephridia of Annelids. Since that time the idea that the antennal gland is in part mesodermic has gradually gained ground, but the question is still treated in current writings as in a degree an open one, though nearly all the text-books, following Reichenbach, state that it is entirely ectodermic in origin.

No one has repeated, as far as I know, Reichenbach's work on the development of the gland in Astacus. With the exception of the work of Boutchinsky ('95),[1] — which unfortunately is little known because of its being in Russian, — no satisfactory account of the embryology of the antennal gland in any Malacostraca has appeared since Kingsley ('89) wrote. It seems probable, in view of the agreement of Kingsley, Boutchinsky, and myself, that Reichenbach's figures and descriptions are misleading, and that he entirely failed to see the endsac. Lebedinski ('92, p. 231), however, endeavors to reconcile Reichenbach's results with those of Kingsley, and with his own on the shell gland of Eriphia, by holding that the mesodermic sheath described by Reichenbach ('86, p. 98) is homologous with the mesodermic constituent forming the endsac in Crangon and in Eriphia. I do not think that Lebedinski's point is well taken, because in Crangon, Eriphia, Gebia, and Homarus the endsac appears before the ectodermic invagination, whereas in Astacus — accepting for the moment Lebedinski's interpretation — it arises much later. Further, the mesodermic sheath described by Reichenbach is, according to his own words, only an enveloping sheath of connective-tissue elements and not at all glandular. This sheath is recognizable in Homarus, where it becomes in part the investing sheath of the gland proper and of the vesicle, and in part the tissue of the blood vessels, but has no part whatever in the formation of the secreting epithelium.

We now know that the antennal gland is of double origin — mesodermal and ectodermal — in Crangon (Kingsley), Gebia (Boutchinsky), and Homarus. These, to be sure, are all in a rather limited group, the Macrura; but it is my belief that similar conditions will be found in other Malacostraca.

The ontogenetic development of the nephridia of Annelids has received attention from many investigators since the pioneer work of Kowalevsky ('71). The stimulation to these researches in the earlier part

[1] The preliminary paper was published a year earlier, Boutchinsky ('94).

of the period came from the problematical nature of the organ, and to this was later added the interest occasioned by the prominent place given this system of organs in the discussion of the Annelid ancestry of Vertebrates.

Since no one has held that the endoderm takes part in the formation of the nephridia in Annelids, the organ presents three possibilities as to origin: (1) that it arises entirely from the ectoderm; (2) that it is wholly mesodermic; or (3) that both ectoderm and mesoderm contribute to its formation. The first of these possibilities has not been advocated by any one, although Wilson ('89) [1] comes very near to such an interpretation. He says (p. 423) that the nephridia "arise in connection with a continuous cell-cord of ectoblastic origin. . . . Each nephric cord terminates behind in a pair of teloblasts derived from the ectoblast. The entire nephric cord is formed by the continued divisions of these 'nephroblasts,' which agree precisely with the 'neuroblasts' in structure, action, and mode of origin." The nephridium (p. 424) is later invested by a sheath of mesoblastic cells which become the peritoneal investment. Wilson believes that the funnel and the investing cells alone arise from the mesoblast, i. e. separately from the rest of the nephridium, and his conclusions are in the main in agreement with those of Whitman ('87, p. 161) in the case of Clepsine. Wilson (p. 426) admits that, in view of the observations of Vejdovský, he cannot "deny the possibility that the glandular part *may* be differentiated from the somatic mesoblast at a very early period, fusing immediately with the cells of the nephric cord, which *may* give rise only to the end vesicle." If this be admitted, his results agree in general with those of Vejdovský (see p. 194).

The other extreme, represented by the second possibility, is reached by Bergh ('88). He finds (pp. 226, 227) in Criodrilus a single funnel-cell differentiated at the point of origin of the septum, from which is proliferated a cord of cells extending posteriorly. This is the beginning of the segmental organ, which soon becomes covered with a sheath. The external terminal part (Endstück) is in no way connected with the epidermis (p. 229), the segmental organ arising entirely from the "Hautmuskelplatte." The same condition is found by Bergh ('90, p. 501) in Lumbricus, in the development of the nephridia in which "Trichter-, Schlingen- und Endabschnitte differenziren sich aus einer einheitlichen Anlage heraus, die in den inneren Muskelplatten ohne Betheiligung der Epidermis entsteht." Bergh finds support in Lehmann's ('87, p. 348)

[1] I refer to his later paper only, as it includes all the essentials of the earlier (Wilson, '87) one.

statement that the epidermis takes no part in the formation of the gland, but concedes ('88, p. 229) that in certain Hirudinea the Endstück arises by an invagination of the epidermis.

The middle ground between these extremes is the position taken by Vejdovský ('84 and '92). In early stages of Rhynchelmis ('84, p. 123), on the posterior face of the dissepiment there is a large cell, from which there is proliferated posteriorly a solid cord of cells. These cells later form a double row and acquire a lumen. This becomes the glandular portion of the organ, which therefore arises from the mesoblast. The nephrostome appears independently on the anterior face of the dissepiment, and later joins the glandular portion by piercing the dissepiment. The cord of cells proliferated posteriorly from the dissepiment is met, at a later stage, by an ectoblastic invagination in the form of a solid cord. From this invagination is derived the lining of the end vesicle and the efferent duct. This ectoblastic lining is, at a subsequent period of development, surrounded by mesoblastic tissue in the form of a sheath. In his later paper ('92, pp. 339–342) he reaffirms the results of his earlier publication. This general plan of development, with slight modification of details, holds, in his opinion, for the nephridia of all Oligochæta ('84, p. 123), as also for Polychæta and Hirudinea ('92, p. 357).

Vejdovský's results are in agreement with those of Kowalevsky ('71) and Boutchinsky ('81), and in part are confirmed by the work of several other writers. It will be noticed that they do not depart widely from Bergh ('88), except in the conditions at the peripheral end.

After comparing these various papers it seems to me that Vejdovský's descriptions and figures are more convincing of accuracy of observation and reliability of interpretation than are those of the writers who adopt one or the other of the extreme views; and yet it is not permissible to disregard the work of such able investigators as Bergh and Wilson. If we except the positive statements of Bergh, which lack the corroboration of later investigators, we may conclude that the evidence goes far to show that both mesoderm and ectoderm share, though unequally, in the development of the nephridium in Annelids, and therefore that, as far as origin from germ layers is concerned, there is no insurmountable obstacle to homologizing the nephridia of Chætopod Worms with the antennal glands of Macruran Crustacea.[1]

[1] Postscript. — Bergh ('99) has repeated Vejdovský's work on Rhynchelmis, but disagrees with him in regard to the conditions and the interpretation to be put on them: "Nach alledem meine ich meine ursprüngliche These, dass Trichter-, Schlingen- und Endabschnitt bei den Oligochäten aus einer einheitlichen Anlage hervorgehen, auch für Rhynchelmis festhalten zu müssen" (p. 446).

Granting, then, that in the antennal glands of Macrura and in the nephridia of Chætopoda both ectoderm and mesoderm are involved, can these organs be compared part for part?

The chief point is to determine in each case the boundary between the ectodermic and mesodermic constituents in the two sets of organs. Where is this in the antennal gland of Macrura? In Homarus, as I believe I have shown, it lies where the lumina of the endsac and of the ectodermal sac (labyrinth) become confluent. Kingsley's results ('89, pp. 29, 30, Figs. 61, 74) in Crangon seem to me to be capable of the same interpretation, the mes-ectal line being at the junction of endsac and ectodermal sac (canal). Boutchinsky's ('95) results on Gebia, since they do not go beyond the earlier embryological stages, are not so precise, but I find nothing in his descriptions or figures irreconcilable with my conclusion, that the epithelium lining the endsac is also of mesodermic origin, while that lining the labyrinth is derived from the ectoderm.

There is difference of opinion as to the corresponding boundary in the nephridia of Annelids. In the permanent nephridia of Rhynchelmis, as well as all other Annelids, according to Vejdovský ('84 and '92), the lining of the efferent duct and of the contractile endsac is, as has been said, ectoblastic, whereas all the glandular part and the nephrostome are mesoblastic in origin. Wilson ('89, p. 425), as we have seen, concludes that the nephrostome and the investing peritoneal sheath of the glandular portion are alone mesoblastic, whereas the epithelium lining the glandular portion, the duct, and the end vesicle are ectoblastic, arising from the "nephric cord." Finally, Bergh ('88, p. 230, '90, p. 501) concludes that in Criodrilus and Lumbricus the entire organ is mesoblastic, there being no ectoblastic constituent whatever. Thus according to Vejdovský the mes-ectal line is at the junction of the glandular region and the efferent duct; according to Wilson it is at the base of the nephrostome, and according to Bergh the gland presents no such line.

It seems impossible to draw any satisfactory conclusion from this conflicting evidence. These differences of opinion are not solely explicable upon the assumption of differences of methods in accomplishing the result in different worms, for all of these observers have worked and based their conclusions in part upon Lumbricus. The only point of complete agreement is that the nephrostome is mesoblastic.

If it is impossible to determine so fundamental a question as where mesoblast ends and ectoblast begins, it is idle to attempt more detailed comparisons. In the present unsettled state of knowledge as regards the

origin of the nephridia of Annelids, the most that can be said is, that the endsac of the antennal gland in Crustacea may be homologous with the nephrostome of the nephridium in Annelids, together with perhaps a part or all of the nephridium peripheral to the base of the nephrostome.

In some points there is considerable difference between the nephrostomic end of a nephridium in an Annelid and the endsac of an antennal gland in Macrura. The lumen of the former is usually in direct continuity with the cœlom, and is ciliated; the latter is closed and non-ciliated. After the work of Grobben ('79) on the shell gland, this question occasioned considerable discussion, and it was claimed that these differences must invalidate any proposed homology between the two organs; but later investigation has shown that the blood sinuses surrounding the endsac of the antennal gland are not homologous with the nephrostomic spaces in Annelids, and therefore the premise upon which this discussion was based is destroyed.

Eisig ('87) holds that the nephridium of Annelids has two functions: (1) the elimination of solid particles from the cœlomic spaces, a duty performed by the nephrostome and in which the cilia take an active part; and (2) the excretion of soluble products from the blood and cœlomic fluid, a function exercised by the excretory cells. If this view be correct, — and there are many things which support it, — the nephrostomic function is not directly represented in the antennal gland of Crustacea, and we therefore cannot expect close morphological resemblance. Hence it seems probable that the closed endsac, in place of an open nephrostome, does not necessarily invalidate a general homology between the two sets of organs.

B. The Number of Metameric Organs of this Nature in Crustacea.

Thus far I have considered the glands in Crustacea from one segment only, — that of the second antenna. In Annelids the nephridia are repeated through a relatively large, although varying, number of segments. Is such metameric repetition realized in Crustacea?

Boutchinsky ('95, p. 170, Tab. VI. Fig. 145, Tab. VII. Figs. 157, 162) has described in the first maxilla of Gebia the development of a mesodermic structure which takes on the form of a tubule closely resembling the antennal gland in histological detail, inclusive of staining qualities. Only the earlier stages of this organ are described, but from this evidence the author thinks the organ is probably excretory, and that it belongs to the same series as the antennal gland.

The shell gland of the second maxillary segment has long been considered as representing an Annelid nephridium, both from its resemblance in function to that organ and from the fact that it is derived from both ectoderm and mesoderm. This gland is widely distributed in Crustacea. It is known as an adult organ, the shell gland, in many Entomostraca and some Malacostraca; moreover, an organ similar in development and appearance is found during embryonic and larval life in the basal segment of the second maxilla of certain Malacostraca, although it disappears before the adult stage is reached. As a *larval* organ the shell gland has been described in the fifth segment of Palæmonetes (Allen, '93[a]) and in some Schizopods and Decapods (Claus).

Thus in many Entomostraca and Malacostraca glands closely resembling one another are developed in both the second (antennal) and fifth (second maxillary) segments; but in some cases — chiefly Entomostraca — that of the fifth segment remains as an adult organ (shell gland), while that of the second segment atrophies. On the other hand, in most of the higher Malacostraca, it is the organ of the second segment (antennal gland) which persists in the adult, while the organ of the fifth segment, if it appears at all, degenerates before adult characters are assumed.

According to the comparative anatomical work of A. Dohrn, Claus, and Grobben, the shell gland is composed of the same parts as is the antennal gland.

The development of the shell gland in the different families of Crustacea has been treated of by several authors. It appears that the gland arises from two sources; the mesodermic part forms the endsac, the ectodermic portion the "canal." Hence it agrees in the main with the development of the antennal glands. We may therefore conclude that the antennal and shell glands in Crustacea are serially homologous organs, being similar in structure and development.

Lebedinski ('89, p. 197, Tab. III. Figs. 79–81, '90, p. 184) describes in Eriphia spinofrons the development of a "Segmentalorgan" as an evagination of the somatopleure, which becomes tubular and grows forward until it ends blindly within the base of the first maxilliped. There arises concurrently an ectodermic invagination in the wall of the coxopodite of this appendage, which meets the evagination from the somatopleure, and their lumina become continuous. We have, then, in this Crustacean a gland in the *sixth* segment, which is apparently of the same series as the antennal and shell glands.

The branchial glands of Crustacea were first noted in Crayfish, Crabs,

and Paguridæ by Cuénot ('87, p. xlv), but were considered as of a lymphatic nature. In his later and more complete work Cuénot ('91, pp. 76–80) describes these glands in several Decapods as found between the two branchial blood channels (Crabs and Pagurus), or as closely investing the walls of either the efferent vessels (Palæmon), or of the afferent vessels. He still ascribes to them lymphatic functions, and makes no mention of Kowalevsky's paper, which had appeared two years before.

Kowalevsky ('89) studied these organs from a physiological point of view. He found (pp. 35–42) that by *intra vitam* injection of a one per cent solution of ammonium carminate into the body spaces of Astacus, the endsac of the antennal gland soon became red. If indigo-carmine be injected, the labyrinth becomes colored blue, but the endsac remains uncolored; and if a thorough mixture of these two pigments be used, the endsac and labyrinth rigidly select the colors. Injection of tincture of litmus gave reactions showing the endsac to be acid, the labyrinth in part alkaline and in part neutral. Palæmon yielded almost identical results, as far as regards the antennal glands, but in addition there were produced by the injection of tincture of litmus two red streaks in each gill, one on each side of the shaft. There were eight double streaks on each side of the body, viz. on the five gills and the appendages of the three maxillipeds. This coloration was caused by rows of cells in which were the same color appearances as in the cells of the endsac of the antennal glands. Further, these cells acted toward the mixture of ammonium carminate and indigo-carmine precisely as did the cells of the endsac, from which it is to be inferred that there is in each gill tissue of the same function as that of the endsac. These glandular regions are the so called branchial glands.

The purely physiological work of Kowalevsky was supplemented by Allen ('92, p. 79), who describes the structures of these glands in the gills of Palæmonetes. They are spherical and composed of conical cells, the apices of which border a central area. There are two varieties of the gland cells, somewhat different in shape and in position. Allen tentatively considers these spherical branchial glands as belonging to the series of ectodermal glands. It should, however, be noticed that, according to Cuénot and Kowalevsky these glands agree in function with the mesodermic portion of the antennal glands.

The description and figures given by Allen strongly remind one of the tegumental glands described by Herrick ('95, p. 125, Plates A and 49) from the pleopods of the female Homarus, the function of which is the

secretion of the glutinous material by which the eggs are fastened to the hairs on the pleopods. Glands of the same general structure are of wide distribution in the integument in Homarus and other Crustacea, as well as in the alimentary tract. Lang ('89–'94, p. 338) says that the dermal glands of Crustacea take part in excretion.

It is still questionable whether the branchial glands are modified tegumental glands, or are segmental and therefore belong to another category. The physiological evidence points strongly to their being nephridial organs, and this is strengthened by the fact that Cuénot ('94, p. 249) has found in the branchial glands chemical products closely resembling those which, according to Marchal ('92), are found in the antennal glands. In histological structure, too, they resemble closely the endsac of the antennal gland. They possess, however, no duct to the exterior, and Cuénot suggests that the products elaborated in these glands may, without chemical change, be carried by the blood to the antennal glands there to be eliminated.

Boutchinsky ('95, p. 168, Tab. VII. Figs. 163, 164) has described the development by invagination from the ectoderm of certain glands whose ducts lead to the gill chamber. These lie in the gill cavity, commonly in the dorsal wall, and he considers them as belonging in the same series as the branchial glands. But even if these are to be classed with branchial glands, they certainly are not, like the branchial glands, homologous with the endsac of the antennal glands, but rather with its labyrinth. I believe, however, that Boutchinsky has erred in classing these with the branchial glands of Cuénot, Kowalevsky, and Allen, for they seem to me to belong rather to the category of tegumental glands.

The position of the branchial glands in the axis of the gill, and in close relation to the blood vessels, as described by Cuénot and Allen, leads one to believe them to be mesodermic rather than ectodermic structures, and with this their physiological activities are in harmony. They have no duct as far as known, and there are several of them in a single gill. If in each gill they represent a single segmental organ, this organ must be in a diffuse condition.

So far as the meagre evidence goes, it shows, in my opinion, that the branchial glands belong to the same category as the antennal and shell glands, but that they represent only the mesodermic parts (endsac) of these. It must, however, be confessed that the evidence for this conclusion is not very satisfactory. A careful examination of the development of the branchial glands will go far toward establishing

or invalidating this homology. I hope soon to undertake such a study.

Kingsley ('89, p. 32, foot-note) has suggested that the genital outlets represent part of a metamerically repeated series of ducts. The female and male genital openings in Decapods, for example, occupy the bases of the eleventh and thirteenth pairs of appendages respectively; their similar position is, in Kingsley's opinion, "inexplicable upon any other ground than that the oviducts and *vasa deferentia* are themselves modifications of pre-existing metameric organs, and the only organs in the Annelids which would answer the requirements of the case are the nephridia."

The first part of his proposition is strengthened by evidence given by Bateson ('94, pp. 152–155), who found that in over three per cent of the females of Astacus fluviatilis examined, there appeared supernumerary oviducal openings. These were either bilaterally symmetrical, or on one side only, and were not necessarily situated in the segment adjacent to the normal opening, but were sometimes removed to the thirteenth segment. Dissection showed that, in cases where such supernumerary openings appeared, the oviduct was branched and communicated with all the openings, which were presumably functional. Oviducal openings were found on the eleventh (normal), twelfth, and thirteenth segments, but no such repetition of genital openings was noted in males. It is probable, then, that Kingsley's proposition that the genital ducts are members of a metameric series is valid; but whether or not they are homologous with the nephridia of Annelids is quite another question. There are in Annelids other metameric organs of somewhat similar position, of which these ducts in Crustacea might be representatives, e. g. the setigerous glands or the dorsal pores. The fact that in Annelids the genital products are in many cases carried to the exterior by the nephridia, or by modified nephridial ducts, lends support to Kingsley's contention; but we cannot rely solely upon the evidence of analogy from which to draw conclusions as to homology.

The evidence on the ontogeny of the genital ducts and their openings in Crustacea is not very complete, but in the main it points to their peripheral portions at least as being ectodermic invaginations entirely distinct from the primary reproductive organs. If this be confirmed, it will add support to the view that they are homologous with the ectodermic portion of the nephridium of Annelids; but it must be remembered that the setigerous glands of Annelids also have a similar origin.

It seems possible that these ducts may hereafter be shown to represent the ectodermic part of nephridia, while the branchial glands represent the mesodermic portion, in which event the homologs of the entire nephridium would exist in the eleventh and thirteenth segments in Decapods, though the ectodermic and mesodermic portions do not come into conjunction as in Annelids; and though, further, the mesodermic constituent is separated into several masses. Such a scheme must remain purely hypothetical until the conditions can be thoroughly studied from the standpoints of both development and comparative anatomy.

To sum up the foregoing discussions, I believe that there is sufficiently good evidence that representatives of the nephridium of Annelids are found in Crustacea in the somites bearing the second antenna (antennal gland), the second maxilla (shell gland of Entomostraca and some Malacostraca), and the first maxillipeds ("Segmentalorgan" of Lebedinski); and, further, that there is partial evidence of such representatives in the somites of the first maxilla (Boutchinsky), and in the eight thoracic somites of the maxillipeds and pereiopods of Malacostraca, namely, the branchial glands which exist in all these somites, and the genital ducts which are found in part of them.

Summary.

1. The antennal gland consists of three parts: gland proper, vesicle, and duct.

2. The gland proper consists of endsac (dorsal) and labyrinth (ventral), whose lumina are connected by a *single* small orifice, which is in the lateral anterior part of the organ.

3. The lumen of the endsac is connected with the exterior only indirectly, by way of the labyrinth.

4. The labyrinth leads into the duct by a series of converging canaliculi, which open by separate pores through the dorso-median wall of the duct. These canaliculi form the "white lobe," which projects ventrad from the median anterior region of the labyrinth.

5. The duct is short and opens through a tubercle on the ventral side of the coxopodite of the second antenna. The opening is guarded by an operculum.

6. The vesicle is a dorsal diverticulum from the duct, and has no direct connection with the gland proper.

7. The blood supply of the organ is from the antennal and sternal arteries.

8. The nerve supply is from the antennal nerve.

9. The endsac encloses a single chamber with numerous radiating outpocketings; the labyrinth is a complicated mass of anastomosing tubules, which are short and of varying calibre.

10. The epithelial cells of endsac and labyrinth are of distinctly different types, and there are no cells of an intermediate nature.

11. The cells of both endsac and labyrinth take part in the secretion. This is in large measure given off in the form of globular vesicles containing an irregular granular mass or clot; these are constricted off from the free ends of the cells.

12. The wall of the vesicle is made up of a single layer of epithelium without folds, surrounded, except on the ventral side, by a muscular sheath, which is variable in thickness. It is presumably by the contraction of this sheath that the secretion is forced out of the external orifice of the duct. Between the epithelial and muscular layers there is a vascular layer.

13. The endsac arises from the mesoderm occupying the axis of the second antenna, when the embryo is 15 to 17 days old. At first there are but one or two cells differentiated.

14. By nuclear division without corresponding formation of cell walls there is formed a *solid* multinuclear body, — a syncytium.

15. Vacuoles appear in the syncytium and probably by confluence form a single large vacuole.

16. The belated cell walls form around this vacuole, so that the vacuole becomes an intercellular space, — the lumen of the endsac.

17. The ectodermic ingrowth — which in the adult becomes the labyrinth — occurs on the median ventral face of the antenna, and appears when the embryo is 28 to 30 days old.

18. This ingrowth passes, first, proximad and then around the anterior side of the endsac; by separation of its cells a lumen is formed near its deep end.

19. The separation of cells progresses rapidly toward the exterior, which is reached by the end of the sixth week. The lumen thus formed becomes the permanent duct and the lumen of the labyrinth.

20. The lumina of the endsac (mesodermic) and of the ectodermic sac (labyrinth) do not become continuous until the embryo is 273 to 303 days old.

21. The adjacent walls of the two sacs break through at a single point. There is consequently an abrupt transition — marked by the mes-ectal line — from the mesodermal to the ectodermal part of the organ, the epithelial cells of the two parts being from the beginning of different types. This boundary persists in the adult.

22. The organ probably does not secrete until after hatching.

23. At hatching the lumen of the ectodermic sac increases greatly in size.

24. In the first larva the endsac is for the most part ventral to the ectodermic sac. The duct leads from the ventral region of the ectodermic sac to the exterior.

25. At this stage the cells of both endsac and ectodermic sac (labyrinth) show evidence of secretory activity. The lumen of the ectodermic sac alone contains a granular coagulum.

26. The epithelial cells of the ectodermic sac in the first larva have a vertically striate cuticuloid layer on the free end. Such a structure is not present in any cells of the endsac.

27. At this stage the epithelium of the duct differs from that of the ectodermic sac in having neither a striated cuticuloid layer nor striated cytoplasm, but there is a gradual transition from one kind of epithelium to the other.

28. The gland of the second larva differs from that of the first chiefly in size, which increases proportionally to the increase in the size of the larva.

29. The development of evaginations from the ectodermic sac (labyrinth) begins in the third larva. In later larvæ these evaginations increase in number and size, and form anastomoses. Thus the complications of the adult labyrinth result from evagination and anastomosis, not from the coiling of a single tubule.

30. Secretion by globules (as in the adult) begins in the third larva and increases in amount in older larvæ.

31. The vesicle first appears in the third larva, as a large, open dorsal evagination from the anterior portion of the ectodermic sac. In older larvæ it increases rapidly in volume and grows caudad.

32. During larval life the chief growth of endsac and ectodermic sac are in different directions. The result is that the two parts of the gland revolve around an approximately antero-posterior axis, so that the endsac comes to lie, as in the adult, dorsal to the ectodermic sac.

33. The conflicting evidence upon the development of the nephridium in Annelids makes it impossible closely to homologize this structure and the antennal glands of Macrura. The endsac may be homologous with the nephrostome of the nephridium of Annelids together with (*a*) none, (*b*) a part, or (*c*) all of the remainder of the organ, depending upon which is the correct view in regard to the development of the nephridium in Annelids.

34. The nephridium of Annelids is *probably* represented in Crustacea in the second (antennal) segment by the antennal gland of Malacostraca; in the fifth (second maxillary) segment by the shell gland of Entomostraca and some Malacostraca; in the sixth (first maxillipedal) segment of *some* Malacostraca by the "Segmentalorgan" of Lebedinski; it is *possibly* represented in the fourth (first maxillary) segment by the excretory organ described by Boutchinsky, and in the sixth to thirteenth (maxillipedal and pereiopodal) segments in part by the branchial glands, and in part (in the eleventh and thirteenth segments) by the genital ducts.

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EXPLANATION OF PLATES.

All figures were outlined with the camera lucida.
The orientation of figures is given for each plate separately.

ABBREVIATIONS.

art. sac.	Sacculary artery.
at. 1.	First antenna.
at. 2.	Second antenna.
cl. lby.	Cells of the labyrinth.
cl. sac. trm.	Cells of the endsac.
cœl.	Body cavity.
cry.	Crystals.
cta.	Cuticula.
drm.	Dermis.
ec'drm.	Ectoderm.
evag.	Evagination.
fas. ax.	Axillar bundle.
fos.	Depression.
glb.	Globule.
gl. e'drm.	Tegumental gland.
gn. opt.	Optic ganglion.
gn. su'œ.	Superœsophageal ganglion.
hi.	Hilum.
i'vag. ec'drm.	Ectodermic invagination.
lac. sng.	Blood lacunæ.
la. cx.	Non-pigmented calcified layer of the shell.
la. cx'.	Pigmented calcified layer of the shell.
la. e'th.	Epithelial layer.
la. mu.	Muscular layer.
lby.	Labyrinth.
lig.	Ligament.
ln. mes-ec.	Mes-ectal line.
lob. l-a.	Lateral anterior lobe.
lob. m-a.	Median anterior lobe.
lob. p.	Posterior lobe.
lu.	Lumen.
marg.	Border.
mb. ba.	Basement membrane.
md.	Mandible.
ms'drm.	Mesoderm.
mu.	Muscle.
mx. 1.	First maxilla.
oc.	Eye.
of. ex.	External orifice.
of. i.	Internal orifice.
op.	Operculum.
par. cl.	Cell wall.
par. vs.	Wall of vesicle.
sac. ec'drm.	Ectodermic sac.
sac. trm.	Endsac.
set. sns.	Sensory hairs.
tis. con't.	Connective tissue.
vac.	Vacuole.
vas. sng.	Blood vessel.
v/v.	Valve-like fold.
a.	In Fig. 2, marks junction of walls of endsac and labyrinth; in Figs. 47,49, marks region of communication between lumina of endsac and ectodermic sac.

PLATE 1.

(In Fig. 1 anterior is up on the plate ; in Fig. 2 the dorsal edge of section is up ; in Fig. 14 the ventral edge of section is down, the posterior edge to the right.)

Fig. 1. Dorsal view of right gland of adult with greater part of vesicle removed. × 2. (Drawn by Mr. K. Hayashi.)

Fig. 2. Anterior face of a transverse section through left gland of adult about ½ mm. posterior to hilus. × 14.

Fig. 3. Section of a fold in the floor of the endsac, together with adjacent walls of labyrinth tubule. × 350.

Fig. 4 Cells, showing globules, from wall of endsac. × 410.

Fig. 5. Cells, containing crystals (see Fig. 6), from wall of endsac. × 410.

Fig. 6. Crystals (probably artifacts, see p. 164 of text), from cells of endsac wall. × 410.

Fig. 7. Section of fold in wall of a labyrinth tubule showing formation of globules. × 410.

Fig. 8. Section of wall of labyrinth tubule showing striate cuticula of epithelial cells. × 410.

Fig. 9. Surface view of epithelium from posterior region of vesicle. × 410.

Fig. 10. Transverse section of wall of vesicle through a region where muscular layer is thick. The muscle cells are cut obliquely. × 410.

Fig. 11. Transverse section of wall of vesicle through a region where muscular layer is thin. × 410.

Fig. 12. A single muscle cell teased from wall of vesicle. × 410.

Fig. 13. Region of nucleus from a cell similar to that of Figure 12. × 900.

Fig. 14. Lateral face of a proximo-distal section through left second antenna in the region of the tubercle on which the duct opens. × 27.

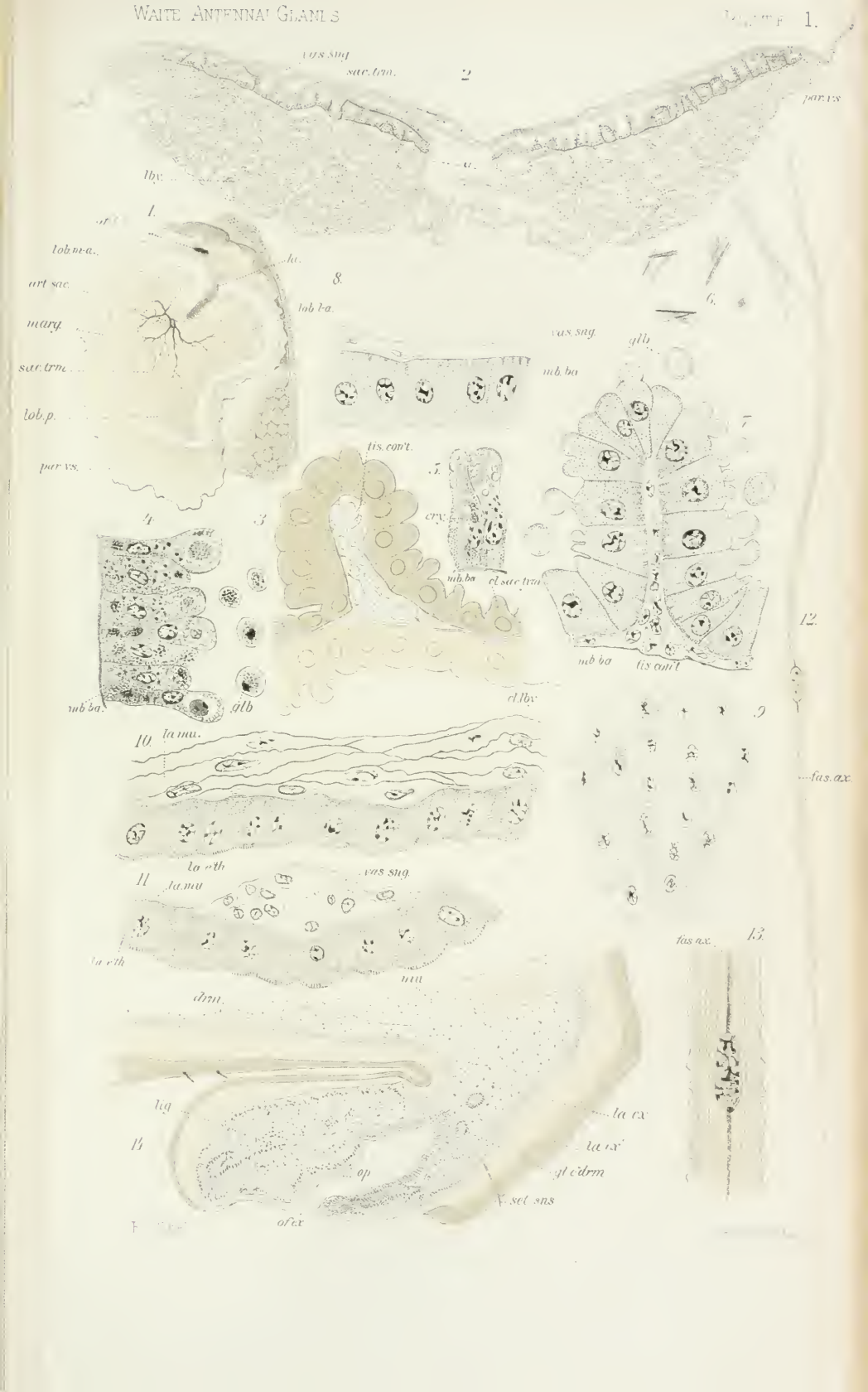

vas.sng
sac.trm.
2
par.vs
lby.
1.
lob.m-a.
la.
8.
art.sac.
lob.l-a.
marg.
sac.trm.
lob.p.
par.vs.
vas.sng.
glb.
mb.ba
6.
tis.con't.
5.
7.
4.
3.
crv.
mb.ba
el.sac.trm
12.
mb.ba
glb
el.lby
9
10
la.mu.
fas.ax.
la.eth
11
la.mu
vas.sng.
fas.ax.
13
la.eth
mu
drm.
lig.
la.cx
la.cx'
op
gl.drm
V.set.sns
of.cx

PLATE 2.

(In Figs. 15–17 anterior is up on the plate; in Figs. 18–24 (except 23) dorsal is up; in Fig. 23 lateral is up; in Figs. 18, 20, 22, 24, anterior is to the right; in Figs. 19, 21, 23, anterior is to the left; in Fig. 25 mediad is to the right. In Figs. 18–25 the mesoderm is distinguished by the darker shade.)

Fig. 15. Ventral view of an embryo 15–17 days after extrusion of egg. × 72.

Fig. 16. Similar view of an embryo 16–18 days after extrusion of egg (stage at which gland first appears). × 72.

Fig. 17. Similar view of an embryo 23–25 days after extrusion of egg. × 72.

Fig. 18. Part of parasagittal section through second antenna of embryo 15–17 days old, showing undifferentiated mesoderm in axis of antenna. × 410.

Fig. 19. Similar section of embryo 16 to 18 days old (Fig. 16), showing (in the mesoderm) first differentiation of endsac with two nuclei. × 410.

Figs. 20–22. Parasagittal sections through antenna at slightly more advanced stages. (Fig. 20 is youngest, Fig. 22 oldest.) × 410.

Fig. 23. Section inclined slightly to the frontal plane at a stage when the endsac is ovate in form and contains in all 12 nuclei. × 410.

Fig. 24. Parasagittal section through antenna in which a vacuole appears in the endsac. × 410.

Fig. 25. Anterior face of a transverse section through right antenna at stage when invagination of ectoderm first begins. (Embryo 28–30 days old.) × 410.

16.
oc.
at.1.
ms.1
at.2
15.
oc.
at.1.
at.2.
mid.
17.
oc.
at.1.
at.2.
23.
ms'drn
ec'drn
sac.trn.
19.
parel
ms'drn
ec'drn
25.
sac.trn
v.vag.ec'drn.
ms'drn.
ms'drn.
parel
sac.trn
ec'drn.
mid
21.
18
ms'drn
ec'drn.
at.1.
mid.
20.
sac.trn.
at.1
ms'drn.
ec'drn
22
sac.trn.
ec'drn.
at.1.
ms'drn
24.
vac
sac.trn.
ec'drn
at.1

PLATE 3.

(Dorsal is up on the plate in all figures. In Figs. 26, 27, 28, 30, anterior is to the right; in Figs. 29, 31, median is to the right. Mesoderm is distinguished by the darker shade.)

Fig. 26. Lateral face of a parasagittal section through left antenna of embryo, showing vacuolation in the endsac and the path of the ectodermic invagination. The invaginated cells in Figs. 26, 27, are more deeply shaded than the rest of the ectoderm. × 410.

Fig. 27. Similar section in slightly older embryo, showing expansion at deep end of ectodermic invagination, and a lumen formed in the endsac. × 410.

Fig. 28. Similar section in embryo about 35 days old, showing position of future lumen of ectodermic sac. × 410.

Fig. 29. Anterior face of transverse section through right antenna of an embryo about 40 days old, showing lumina of endsac and ectodermic sac and duct to exterior. × 410.

Fig. 30. Lateral face of a parasagittal section through left antenna of embryo 60–70 days old, showing growth of ectodermic invagination around the endsac. × 410.

Fig. 31. Anterior face of transverse section through right antenna of embryo 3 to 4 months old, showing extension of ectodermic invagination. × 410.

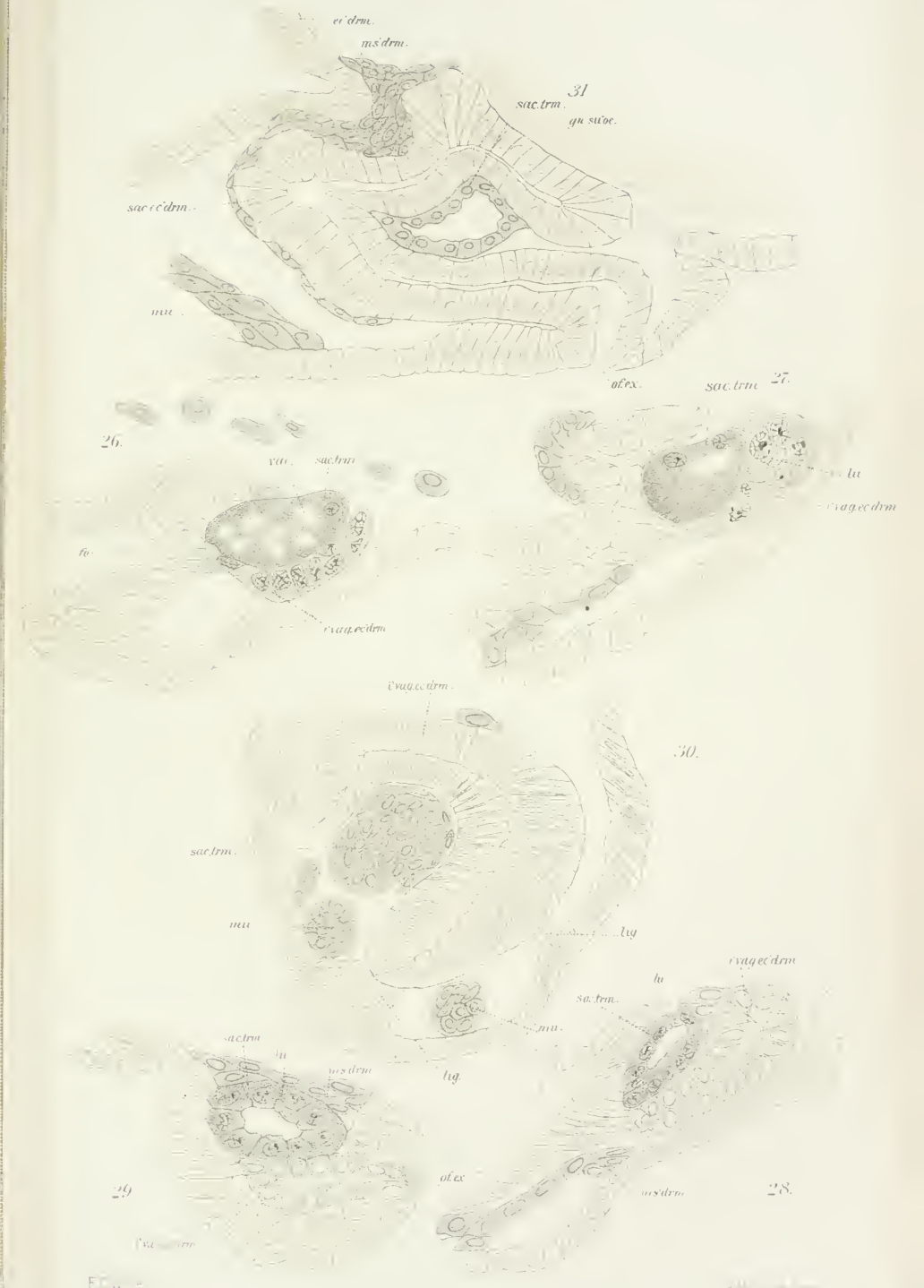

ec.drm.
ms.drm.
31
sac.trm.
gn.suc.
sac.ec.drm.
mu.
ol.ex.
sac.trm.
27.
26.
vag.
sac.trm.
lu.
vag.ec.drm.
fo.
vag.ec.drm.
vag.ec.drm.
30.
sac.trm.
mu.
lig.
vag.ec.drm.
lu.
sac.trm.
mu.
sac.trm.
lu.
ms.drm.
lig.
ol.ex.
ms.drm.
29.
28.

PLATE 4.

(Dorsal is up on plate in all figures. In Figs. 32–38 anterior is to the left; in Fig. 39 anterior is to the right.)

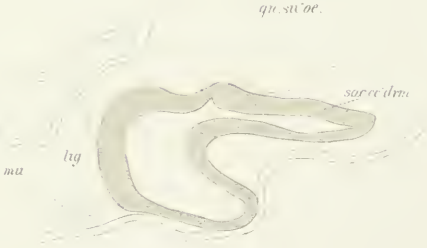
Fig. 32–38. Lateral face of the second, third, fifth, sixth, eighth, eleventh, and twelfth sections respectively from a series of thirteen parasagittal sections through the left antennal gland of the first larva. × 128.

Fig. 39. Median face of a nearly parasagittal section through the left gland and duct of the first larva. × 128.

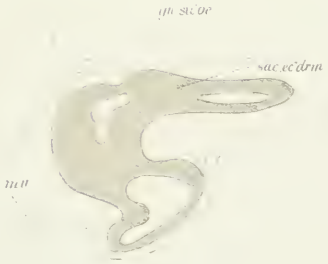
35.
qu.su'oe

36

37.
qu.su'oe.



38



39.

PLATE 5.

(Dorsal is up on all figures. Median is to the right in Figs. 41–48.)

Fig. 40. Anterior face of a transverse section through the region of the base of the second antennæ in the first larva. (The dorso-ventral axis has been slightly shortened in cutting.) × 42.

Fig. 41–48. Anterior face of the third, fifth, seventh, ninth, tenth, twelfth, fourteenth, and seventeenth sections respectively from a series through the right antennal gland of the first larva. × 128.

40.
41
42
43.
44.
45.
46
47.
48
sacrum
sacr.dr.
sacr.tr.
sacr.ca.
sacr.ve.
sacr.ar.
sacr.ax.
sacr.ma.
sacr.cr.
sacr.ce.
sacr.ba.
sacr.ap.
sacr.ob.
sacr.ro.
sacr.ri

PLATE 6.

(In Fig. 49 anterior is up, lateral to the right; in Figs. 50, 51, 60, dorsal is up; in Figs. 50, 60, anterior is to the right; in Fig. 51 anterior is to the left.)

Fig. 49. Dorsal face of a frontal section (the tenth of a series of eighteen) through the right antennal gland. × 128.

Fig. 50. Lateral face of a parasagittal section through right gland and duct in the third larva. × 128.

Fig. 51. Parasagittal section through the orifice connecting the lumina of endsac and ectodermic sac in the first larva. × 350.

Fig. 52. Cell from section of wall of endsac of the first larva in a region not adjacent to the ectodermic sac. × 900.

Fig. 53. Cells from section of wall of endsac in the first larva in a region adjacent to the ectodermic sac. × 900.

Fig. 54. Cells from section of dorso-lateral wall of ectodermic sac in first larva. × 900.

Fig. 55. Cells from section of ventral wall of ectodermic sac in first larva. × 900.

Fig. 56. Cells from section of dorso-median wall of ectodermic sac in first larva. × 900.

Fig. 57. Cells from section of dorsal wall of ectodermic sac in first larva, one showing mitotic figures. × 900.

Fig. 58. Cell from section of dorsal wall of ectodermic sac in first larva. × 1600.

Fig. 59. Blood corpuscle from blood lacunæ of antennal gland in first larva. × 900.

Fig. 60. Longitudinal section of duct of the antennal gland in first larva. × 350.

evag
lac sng
sac ec drm
hg
50.
hg
49
lac sng.
a.
sac ec drm
57
58
mb ba
53
52
59
56
55
vas
coel.
coel
60
of ex
51

THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos.

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[3] XV.[16] The Stalk-eyed Crustacea.

S. GARMAN. The Fishes.

W. GIESBRECHT. XVI.[15] The Copepods.

A. GOËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E. HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

H. LUDWIG. IV.[5] XII.[14] The Holothurians.

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[1] Bull. M. C. Z., Vol. XXI., No. 4, June, 1891, 16 pp.; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates.

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull. M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z., Vol. XXV., No. 1 September, 1893, 25 pp.

[8] Bull. M. C. Z., Vol. XXV., No. 2, December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4, January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C. Z., Vol. XXV., No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV., No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z., Vol. XXV., No. 10, October, 1894, 109 pp., 12 Plates.

[14] Mem. M. C. Z., Vol. XVII., No. 3, October, 1894, 183 pp., 19 Plates.

[15] Bull. M. C. Z., Vol. XXV., No. 12, April, 1895, 20 pp., 4 Plates.

[16] Mem. M. C. Z., Vol. XVIII., April, 1895, 292 pp., 67 Plates, 1 Chart.

[17] Bull. M. C. Z., Vol. XXVII., No. 3, July, 1895, 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol. XXVII., No. 4, August, 1895, 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895, 14 pp., 3 Plates.

[20] Bull. M. C. Z., Vol. XXIX., No. 1, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M. C. Z., Vol. XXIII., No. 1, September, 1897, 92 pp., 15 Plates.

[22] Bull. M. C. Z., Vol. XXXI., No. 5, December, 1897, 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol. XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M. C. Z., Vol. XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[25] Mem. M. C. Z., Vol. XX[illegible], No. 2, November, 1899, 116 pp., 22 Plates, 1 Chart.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, June, 1899, 4 pp., 1 Plate.

PUBLICATIONS

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Bulletin of the Museum of Comparative Zoology
AT HARVARD COLLEGE.
Vol. XXXV. No. 8.

MATURATION AND FERTILIZATION IN PULMONATE GASTEROPODS.

By Henry R. Linville.

With Four Plates.

CAMBRIDGE, MASS., U. S. A.:
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INTRODUCTION.

In the Autumn of 1896 I undertook this investigation at the suggestion of my teacher, Professor Mark, to whom I am under many obligations for helpful suggestions and criticisms.

In the beginning my plan was to study the maturation and fertilization of Limax maximus L. and Limax agrestis Müller, but owing to ill fortune and inexperience I was unable to preserve a sufficient amount of properly prepared material of the desired stages, and consequently I have been obliged to supplement my work with the results obtained

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. 109.

from a study of two species of Limnæa. The results on Limnæa to some extent repeat the most excellent work by Kostanecki und Wierzejski ('96) on Physa, a near relative of Limnæa. However, additional points of interest in regard to chemical phases in the centrosome, together with some facts concerning reduction division in the second maturation spindle, make this something more than a mere repetition of their work.

Since the publication of Professor Mark's monograph on Limax campestris, in 1881, the technique of embryology has been so much developed that a re-investigation of the maturation and fertilization of this genus of pulmonates with special attention to the history of the centrosome seemed likely to yield interesting and important results. The incompleteness of my series of stages in Limax material will, however, necessitate further work on this genus, which I hope to complete at some time in the near future.

By each addition to the already great mass of cytological literature, it becomes more apparent that if the centrosome is a permanent organ of the cell, it is an organ which goes through various and complicated phases. Certain facts impel us to believe that there is a variation in the chemical condition of the centrosome. For example, in some preparations, at a certain stage of maturation, there is no centrosome (nor centriole) to be distinguished as a deeply staining body at the poles of a perfectly formed spindle, whereas in other preparations at apparently the same stage and treated in the same manner, one may observe centrosomes which are deeply stained and of enormous size in comparison with the volume of the cell. This great variation of the centrosome is, however, not inconsistent with the idea of its permanence in the first maturation spindle, where the astral rays always indicate its presence. When, however, no astral rays are visible, the difficulties in the way of identifying the centrosome are exceedingly great, so great in fact as to render it well-nigh impossible to distinguish it from the yolk granules.

Collection of Material.

I have collected and preserved eggs of Limax agrestis, Limax maximus, and of two species of Limnæa. Limax maximus lays in the vicinity of Cambridge, Mass., during October, November, and December. Limax agrestis begins laying in early summer, and may, under especially favorable circumstances, continue to lay late into the winter. The eggs of

Limax maximus may be found in the vicinity of Cambridge in damp, protected places, under rotting wood, or under waste lumber and straw, and especially about greenhouses, where these slugs, and also Limax agrestis, are found most abundantly. The eggs of Limax maximus are not covered with earth, as are those of Limax agrestis. It is seldom that one finds the eggs of Limax agrestis lying exposed on the surface of the ground. Limax maximus in captivity apparently lays its eggs wherever it happens to be; sometimes on the bare side of the box or can in which it is confined; at other times under a piece of rotting wood or other protection. Limax agrestis, on the other hand, almost invariably bores into the loose soil in the box, sometimes nearly an inch, and lays its eggs in a single heap. Its eggs do not cohere as do those of Limax maximus.

In general, early morning is the time when eggs are laid by both species of Limax, although the laying may take place at any time of the day or night. The duration of the time of laying is not great. No continuous observations of the time consumed in laying were made, because of the desirability of getting eggs in as early stages as possible, the animal being killed as soon after the beginning of laying as possible. Considering the time required to extrude several eggs one after another, however, it does not seem likely that more than thirty minutes would be required to complete the extrusion of the largest number deposited at a single laying.

About the 15th of March, 1897, I collected a large number of Limnæa elodes Say, one of the common pond snails. Many pairs were found in the act of copulation. In the course of two days eggs were laid in the aquarium in great abundance. These snails can be stimulated to lay simply by supplying plenty of fresh water and keeping the vessels free from any decaying matter. Laying usually takes place early in the morning, but a sudden change from impure water to pure water will cause them to lay at any time of day.

Technique.

The eggs of Limax were taken either just after laying or from the sexual organs before being laid. The latter were obtained from the uterus, from the albumen gland, or from the oviduct. The eggs just deposited and those abstracted from the uterus, where they lie one after another ready to pass to the exterior, were "shelled," freed from the albumen and fixed. The albumen gland and the oviduct of individuals

which had begun to lay were sectioned; eggs were found in both of these regions.

In freeing the egg cell from the shell and the albumen, I have found the best method to be the one recommended by Kofoid ('95). This consists in placing the eggs, a few at a time, in a watch glass containing normal salt solution, care being taken not to let any eggs remain in the solution longer than ten or fifteen minutes. Taking an egg carefully in a pair of fine forceps, one can either snip the membrane with a pair of sharp-pointed scissors, or, with the aid of a sharp needle, rend it by catching it between one arm of the forceps and the needle. After the egg has escaped through the opening in the egg-shell, the albumen can be washed away from it by a gentle current produced by a pipette. As soon as the eggs are free from albumen they are transferred to the fixing solution. Sometimes I have thrown the entire egg, without shelling it, into the fixing solution, and have subsequently removed the membrane and as much of the albumen as could be taken away with safety to the yolk. The egg membrane must be removed within a short time after the egg has been thrown into the fixing fluid, because otherwise it becomes too hard to be cut successfully. Eggs that have been killed (fixed) without shelling, then washed to remove the killing agent, and dehydrated in alcohol, may be returned through weaker grades of alcohol to water for the purpose of shelling and removing all but a small portion of the surrounding albumen; but far better conditions of yolk and cytoplasm were obtained by removing the albumen from the egg as soon after killing as possible, and usually the result was better still, if the egg was freed from albumen before it was killed. The latter, indeed, is the best of all methods for Limax, but in the case of the eggs of Limnæa, which are quite small, much time can be saved, and apparently quite as good results obtained, by thoroughly fixing the eggs before shelling, providing the egg-shell is removed before the albumen has had time to harden.

For killing and fixing, the following solutions were used: Saturated aqueous solution of corrosive sublimate with 3 per cent to 5 per cent acetic acid, Flemming's fluid, Perenyi's fluid, and three of the mixtures employed by Kostanecki und Siedlecki, viz. (I) a mixture consisting of saturated aqueous solution of corrosive sublimate one part, 3 per cent nitric acid one part, absolute alcohol one part; (II) a solution similar to the last in which acetic acid is substituted for the nitric acid; and (III) a simple 3 per cent solution of nitric acid. Sublimate-acetic is well known for its good preservation of cytoplasmic structures. Flem-

ming's fluid has no advantage over other methods, and has the decided disadvantage of causing very great brittleness. Series of sections, even after being mounted and subsequent to the hardening of the balsam, frequently break into small bits under very slight pressure. Perenyi's fluid was used much more successfully. The excessive brittleness noticed in the Flemming preparations was not present in those made with this mixture; moreover, with Perenyi's fluid nuclear structures were well preserved, and when stained the elements came out clearly. Cytoplasmic structures, including the astral rays, were in most cases sharply marked. The methods proposed by Kostanecki und Siedlecki were not tried with Limax material. In the eggs of Limnæa the nuclear and the cytoplasmic structures were preserved very well by the three methods of Kostanecki und Siedlecki; of the three, the 3 per cent nitric acid solution gave the best results, the preservation by this method being exceptionally good.

Heidenhain's iron-hæmatoxylin was used exclusively in staining. Slides with sections affixed were immersed in the 2 per cent iron-alum mordant for a period varying from three to twelve hours, and after washing in a gentle current of tap water for several minutes, were placed in the $\frac{1}{2}$ per cent aqueous solution of hæmatoxylin and left for periods varying from eighteen to forty-eight hours. A 2 per cent iron-alum solution was used for decolorizing, the process being carefully watched by frequent examination under a low power of the microscope. In most cases the yolk could be decolorized sufficiently to disclose the centrosomes, for example, without decolorizing the centrosomes themselves.

A. MATURATION.

I. General Account.

1. Limax Eggs in the Oviduct.

My study of the eggs of Limax as they occur in the hermaphrodite gland has been very limited. As far as the position of the nucleus in such eggs is evidence, I cannot discover that the egg has any pre-established axis, the nucleus being always central.

The earliest observed stage in the first maturation spindle was seen in eggs found in the oviduct and apparently not long freed from the hermaphrodite gland. Indeed, judging from the proximity of these eggs to the hermaphrodite gland, it seems highly probable that changes

leading to the formation of the first maturation spindle begin before the egg is set free from that organ. The least developed of the eggs found in the oviduct showed the first maturation spindle already established, and nearly the whole of the germinative vesicle involved in the spindle.

2. Limax Eggs in the Albumen Gland.

I found a few eggs in the albumen gland imbedded in a small mass of albumen; there was no trace of an egg membrane. In these eggs the first maturation spindle was in every case completely formed and lying near the middle of the egg. There was no indication of the presence of a spermatozoön in the eggs found either in the oviduct or in the albumen gland.

3. Limax Eggs in the Uterus.

In the uterus of Limax agrestis I found a few eggs in which the first maturation spindle had not yet begun to move toward the periphery. The earliest stage of the eggs of Limax maximus secured was found in the uterus, no eggs of this species having been obtained either in the oviduct or in the albumen gland. In these (L. maximus) eggs the first maturation spindle was eccentric in position, the centre of one centrosphere being near the periphery of the egg. Uterine eggs were kept separate from eggs already laid, and likewise from eggs obtained from the oviduct or the albumen gland, but no note was taken of the exact location of eggs in the uterus, whether they were nearer the albumen gland or the external opening of the uterus.

The eggs of Limax maximus found in the uterus ranged from a stage in which one centrosphere of the first maturation spindle was nearly peripheral in position to a stage in which the first polar cell was completely formed. Since these eggs were found in the uterus of an animal killed while engaged in laying, one is safe in assuming that the earliest stage likely to be found in an egg of Limax maximus already laid is one in which the first polar cell has been formed. Unfortunately, not a sufficient number of eggs of Limax agrestis were preserved to furnish any definite notion of the earliest and latest stages to be found in the uterus of this species. In the few eggs from the uterus that were sectioned and examined, the first maturation spindle was nearly central.

The earliest indication of a spermatozoön within the egg was noted in uterine eggs. The fact that in some cases (L. agrestis) the head of the spermatozoön was still attached to the filament indicates that penetration had taken place only a very short time before fixation by the killing

fluid. In sections of the oldest uterine eggs from Limax maximus (first polar cell completely formed) the head of the spermatozoön — still a single, oval, homogeneous, and slightly swollen body — lies near the centre of the egg, its long axis being directed more or less definitely toward the egg-aster.

4. Eggs of Limnæa.

All the material of Limnæa elodes was obtained from eggs already laid. In the earliest stages thus secured, the first maturation spindle had begun to move from the centre of the egg. In only one instance (Plate 1, Figure 2) have I seen distinct remnants of the germinative vesicle.

II. Centrosome and Centrosphere.

Recent investigation of the nature of the centrosome and the centrosphere has thrown considerable light on the variable nature of these structures. The *centrosphere* cannot at present be considered a permanent structure, but merely a temporary manifestation of an unknown force. As long as the centrosphere appeared to be the region of the beginning of the astral rays, there was good reason for assigning to it a considerable degree of importance. In the light of the recent work on Physa by Kostanecki und Wierzejski ('96), and on Ascaris by Kostanecki und Siedlecki ('96), however, the centrosphere becomes of less significance. They find that the astral rays extend into the centrosphere and even to the centrosome itself. The centrosphere, according to these authors, with whom I agree, is formed merely by a thickening of the rays. Wilson ('96, p. 234) calls attention to the concentric rings of microsomes on the astral rays in the spermatogonium of Salamandra as seen by Drüner, and says that the innermost two rings, being especially prominent, mark off a centrosphere composed of a medullary and a cortical zone. Another case in point which Wilson discusses is that of the rings of microsomes found by Heidenhain in leucocyte asters. The condition shown in leucocytes, with the astral rays beginning at the centrosome (the ultimate structure at the centre), tallies well with what Kostanecki und Wierzejski found in the egg of Physa. If the centrosphere is to be taken out of the category of the permanent organs of the cell, it nevertheless represents a condition in the chemical and physical phases of the cell which is worthy of further investigation.

In the introduction to this paper, I have referred to the variable size and condition of the *centrosome*. This is so intimately connected with

the variation in the centrosphere as to render it advisable, and even necessary, to discuss the two structures together. I shall first take up the results obtained from the study of the maturation of Limax maximus, and then follow with a more complete account of the history of the centrosome and centrosphere in Limnæa elodes.

1. Limax.

(*a*) *First Maturation Spindle.*

The earliest stage of the egg of Limax maximus that I have found is one showing the first maturation spindle fully formed (not figured; compare with next older stage, Plate 3, Figure 16). The spindle, with all the chromosomes in the telophase, has moved half the length of the egg radius toward the animal pole. At either end of the spindle is to be seen a large distinct centrosphere, composed of a central, pale, reticulated area, and a very thick wall. The wall is nearly one third as thick as the diameter of the whole sphere. Figure 16 represents a later stage, but does not differ essentially from the earlier one as far as the condition of the centrosphere is concerned. Careful examination of the centrosphere in both shows that the very fine reticulum of the clear region at the centre is, to all appearances, continuous with the more compact reticulum composing the wall. The astral rays emerge from the outer portion of this wall, but, because of the density of the substance composing the wall, it is impossible to say whether or not the central reticulum is continuous through the wall of the centrosphere with the astral fibres. The thickened wall of the centrosphere and the fine central reticulum are not occasional phenomena, but are present in every first maturation spindle in Limax maximus up to the stage represented in Plate 3, Figure 16. *The most thorough search through specimens fixed by different methods and stained for varying lengths of time has failed to reveal any trace of a centrosome in the kind of centrosphere just described.* Between the stage represented in Plate 3, Figure 16, and the one represented in Plate 3, Figures 17 and 21, is a gap in the condition of the centrosome which I am not able to bridge over by intermediate stages. However, the stages are not so far apart that the changes through which the centrosphere would pass may not be fairly inferred.

Whatever the influence that causes the condition of the centrosphere, it is clear that the process is one of increase in volume of the central finely reticulated area. Figures 17 and 21 are particularly instructive in this regard. In each two centrosomes have made their appearance.

In addition to the fact that in Figure 21 the centrosomes have moved further apart than in Figure 17, the more completely fused condition of the elements composing the "Zwischenkörper" in Figure 21 gives conclusive evidence that it has progressed in development further than Figure 17. I have not been able to follow closely the fate of this central reticulated area after the stage represented in Figure 21.

It will be noticed that in Figure 17 the long diameter of the centrosphere is nearly coincident with the chief axis of the egg, and likewise with a line connecting the two minute centrosomes. In Figure 21, on the contrary, the long diameter of the centrosphere is nearly perpendicular to the chief axis of the egg, and forms a very sharp angle with the line connecting the two small centrosomes. This relation of centrosphere-axis with centrosomes suggests a causal connection, but when compared with the position of the centrosomes in Plate 3, Figure 22, it seems probable that the reason for the variation in the direction of the long axis of the centrosphere, as compared with the chief axis of the egg, in these three cases must be sought in something else than the position of the centrosomes.

Before turning to the consideration of the centrosome, it will be interesting to call attention to the astral rays of Figure 17. Here one set of the astral rays begins at the centrosphere and extends two thirds of the way to the vegetative pole, while another set is composed of very short rays scattered among the long rays. Beginning near the distal end of the long rays and continuing in the same general direction to the periphery of the egg are rather broad, indistinct band-like radiations (unfortunately not well reproduced from the drawing). On examination with a one-twelfth inch immersion lens, the bands were found to be composed of exceedingly fine granules, different from the ordinary yolk granules, and distinct also from the "microsomes" which are visible among the long astral rays. The same faint bands appeared in other eggs of the same stages as those shown in Figures 17 and 21.

There is a point concerning the astral rays in Figure 21, to which I wish to call particular attention, since it may serve to throw new light on the question of the centrosphere. Beginning at the periphery of the central finely reticulated area, the rays extend outward a short distance as a set of extremely fine fibres, then suddenly become thicker, and retain this condition to their peripheral ends. I cannot demonstrate any ring of microsomes at the region of transition from the finer to the coarser fibres, but nevertheless the zone embracing the finer rays is a distinct modification of the aster, and is of interest because of its bear-

ing on the question of the limits of the centrosphere. Other instances of this phenomenon will be referred to further on.

It will be remembered that no centrosome, as that structure is generally understood, is to be made out in connection with the first maturation spindle of Limax maximus. After the formation of the first polar cell, however, we have (in Figures 17 and 21) within the centrosphere the centrosome already divided, presumably in preparation for the formation of the second maturation spindle. In Figure 17 the centrosomes are extremely small, and only one of them gives indication, even with the best immersion lens, of having rays in connection with it, but in Figure 21 the small dense bodies have all the characteristics of centrosomes. Although the centrosomes in Figure 21 are very minute, they may still be made out with certainty at points from which several very delicate rays diverge. These rays may have some connection with rays outside the reticulated area, but if they do, it is only a secondary connection. The rays extending from either centrosome toward the other have united into a very small but fairly distinct spindle (not well shown in the figure).

(*b*) *Second Maturation Spindle.*

My study of Limax maximus points to the conclusion that after the formation of the first polar cell, and possibly during that process, the centrosphere remaining in the egg increases in size to many times the volume it had in the first maturation spindle. Neither after the formation of the first polar cell, nor after the formation of the second, have I been able to trace *continuously* the modification of the centrosphere. In Figure 20, however, the periphery of the centrosphere is very faint; and it seems as if the whole structure were on the verge of disappearance. In a subsequent part of this paper I shall take up the discussion of the fate of the inner centrosphere and also the centrosome of the second maturation spindle.

2. Limnæa Elodes.

(*a*) *First Maturation Spindle.*

My observations on the first maturation spindle of Limnæa elodes have resulted in a number of interesting facts bearing on the relation of centrosphere to centrosome. As stated in the introduction, the centrosome sometimes appears stained faintly, sometimes very deeply. When deeply stained, it varies in size from an extremely small body to one of

the size of the centrosphere itself. The existence of the latter extreme affords strong reason for believing that the entire centrosphere may become stained, for, by a proper serial arrangement of several preparations, one can see that the clear region of the centrosphere surrounding the centrosome is gradually encroached upon by the centrifugal advance of the stainable region, until the entire centrosphere is deeply and homogeneously stained. It is quite possible that the cases of faintly stained centrosomes may be due either to understanding, or, what is more probable, to protracted decolorizing.

In taking up the detailed description of the maturation spindles, I shall follow the order indicated in the discussion of the same structures in Limax maximus; that is, I shall begin with the earliest stage in the first maturation spindle. It will be remembered that in Limax maximus the earliest stage obtained was one in which the condition of the chromosomes of the first maturation spindle already indicated the telophase. That was a uterine egg.

All the eggs of Limnæa elodes were taken after being laid. The earliest stage obtained was that shown in Plate **1**, Figure 2.[1] The spindle is fully formed; it surrounds the disintegrating germinative vesicle, and already has a radial, though deep, position. At either pole of the spindle is a well-developed aster, at the centre of which appears a mass of minute granules, stained yellowish-brown. In the midst of this mass it is possible with an immersion lens to distinguish a very small and faint centrosphere, containing an extremely small centrosome (not to be seen in the drawing). The inner aster is curiously modified by the sperm aster, a phenomenon to which I shall refer later on.

A stage in the egg of Limax agrestis, similar to this, is shown in Plate **3**, Figure 14. In this case the germinative vesicle, judging from the rather uneven arrangement of the chromosomes, has just disappeared. The spindle here is central, and in this respect differs from the condition shown for Limnæa in Plate **1**, Figure 2, where one pole of the spindle is near the centre of the egg. In Figure 14 both the position of the spindle and the condition of the spermatozoön indicate a stage younger than that of Limnæa (Figure 2), but the condition of the germinative vesicles indicates that it is older than Limnæa.

In the eggs of Limnæa I have seen frequently the typical flattening of the animal pole preceding the formation of the first polar cell (Plate **1**,

[1] (Plate **4**, Figure 25, represents the egg of Limax agrestis before it leaves the hermaphrodite gland, and shows the condition of the germinative vesicle at this stage.)

Figure 1). The "polar depression" described by Kostanecki und Wierzejski ('96) for Physa is also not a rare phenomenon in Limnæa. Conklin ('94) suggests that the flattening of the animal pole preceding the formation of the first polar cell is caused by the contraction of the spindle fibres. Perhaps, in a similar way the "polar depression" is produced by a more active contraction of the spindle fibres which lie in the prolongation of the axis of the spindle. But even before the outer centrosphere has reached the cell membrane (Plate 1, Figure 1), the polar depression has disappeared, and a marked flattening of the centrosphere accompanies that of the animal pole of the egg. On either side of the outer centrosphere, as seen in optical longitudinal section of the spindle (Figure 1), there is a projecting "wing" of deeply staining substance. Careful examination shows these wings to be composed of closely crowded astral rays, which have stained near their proximal ends. Since the "wings" are to be seen in all longitudinal sections of this spindle, it follows that the appearance is due to the presence of a continuous disk of staining substance.

Studying the different figures of the first maturation spindle of Limnæa with special regard to the deeply staining portion at the poles, one must, I think, conclude that both centrospheres shown in Figure 1 have taken the stain throughout their whole extent. The roughness of the outline of the stained centrospheres, as compared with those of smaller size, is due to the increasing distance between the astral rays, as one passes outward from the centre. This condition suggests the idea that even parts beyond the limit of the centrosphere may have been stained; for if only the centrosphere were stained, the outline should be more regular. Those investigators who have discovered enormous centrosomes should examine Plate 4, Figure 23. The dense mass shown there at each pole of the spindle looks more like a precipitate lodged about a central point than like an organ of the cell. In Figure 24 the outlines of the centrospheres may be seen. The great irregularity in the form of the stained portions proves beyond question, it seems to me, that the dense masses at the poles of the spindles represented in Plate 1, Figures 1 and 3, and in Plate 4, Figure 23, are simply portions of the cell protoplasm which have not been decolorized.

Many investigators, notably Mark, Garnault, and Kostanecki und Wierzejski, have given in detail for pulmonates the process of the actual formation of the polar cells. It is my purpose in this division of the paper to note only the modifications of the centrosome and centrosphere. In the part devoted to the discussion of the nucleus, I shall make special

mention of the differences in the character of the division of the chromosomes in the first as compared with the second maturation spindle.

Turning now to the question of the centrosome, I find that after the first polar cell has been nearly or quite formed the centrosphere belonging to it, when it can be made out at all, gives evidence of disintegration. This is shown in Plate 2, Figure 8. Nothing which can be maintained to be a centrosome appears in the outer centrosphere of the polar cell. There is, however, in Plate 2, Figure 12, in the only polar cell represented in that figure, a distinct centrosome, with fibres radiating to the chromosomes. The region of the supposed connection of the polar cell with the egg in this section was covered by a mass of foreign substance, so that it is not certain, though highly probable, that the polar cell was joined to the egg. The aster remaining in the egg shown in Figure 8 resembles the condition of the centrosphere shown in Figure 21. In the present case (Figure 8) the centrosphere may be said to be composed of two parts, a very small, central clear area, of spherical form, and a very much larger non-spherical enveloping structure. The two are not concentric, the inner sphere being much nearer to the peripheral flattened wall of the enveloping structure than to its deep rounded extremity. It is to be observed that in Figure 8, at the exact centre of the inner, small centrosphere, there is a minute centrosome, but with no indication of division in preparation for the next maturation spindle. The inner centrosphere, though small, is very distinct. Within this centrosphere no rays could be distinguished; in fact, the contents, under the highest magnification, appeared to be entirely homogeneous. Rather prominent rays, few in number, can be seen passing from the periphery of the inner centrosphere out through the outer or enveloping structure. This outer structure stretches from the plane of the deep ends of the series of chromosomes belonging to the egg, toward the centre of the egg, a distance equal to nearly twice its width. The walls, beginning at the chromosomes, run for a short distance perpendicular to the plane of the chromosomes and then gradually converge and nearly come together, but the lines bounding this outer centrosphere decrease in distinctness in passing from the plane of the chromosomes. The outer structure is to be conceived of as a cylinder, truncate at the peripheral end, dome-shaped at the deep end, and containing a spherical inner centrosphere located much nearer the truncate than the opposite end.

The enlargement of the centrosphere during the completion of the polar cell and after its formation — an occurrence so striking in Limax

maximus — has not yet begun in the specimen from which Figure 8 was drawn. In many other cases, however, at a slightly later stage the centrosphere shows a decided increase in size, as is seen, for example, in Plate 2, Figure 7. The peculiar arrangement of the chromosomes, the disappearing spindle fibres, and the enlarged centrosphere shown in Figure 7 represent a stage I have seen many times. In earlier stages I have never seen the centrosphere as large as it is in Figure 7. This phenomenon of an increase in the size of the centrosphere which has performed its function, was described by Miss Esther F. Byrnes for Limax agrestis in a paper read at a meeting of the American Morphological Society in December, 1896.

b. Second Maturation Spindle.

I have searched with considerable care for evidence of two centrosomes within the enlarged centrosphere of the first maturation spindle of Limnæa elodes, but I have found them in only a single case (Plate 1, Figure 4), a very early stage in the formation of the first maturation spindle. There is no evidence of a spindle forming between the centrosomes, as, indeed, nothing of the kind could be expected at so early a stage. Hence I am unable to say, as far as Limnæa is concerned, what relation the enlarging centrosphere bears to the formation of the second maturation spindle; whether the new spindle is formed *de novo* within the still persisting centrosphere, or whether the centrosphere disappears before the second maturation spindle comes into existence.

A good example of a second maturation spindle near the height of its development is shown in Plate 2, Figure 11. In this case the two astral figures are conspicuously unlike. Frequently I have found in *first* maturation spindles one centrosome differing from the other in size, and also the centrospheres differing in condition, but the variation shown in Figure 11 appears to be of quite another nature. In this case one centrosome, the peripheral one, has no centrosphere surrounding it, and the centrosphere at the deep pole of the spindle is flattened in a plane perpendicular to the axis of the spindle. I have not enough material of the proper stages to allow me to make a careful study of the second maturation spindle with reference to the centrosomes and centrospheres, and hence do not pretend to say whether Figure 11 represents a typical condition.

The phenomenon of concentric centrospheres is not confined to the first maturation spindle. The aster of the second maturation spindle shown in Plate 2, Figure 13, is not altogether like that shown in Figure

8, for Figure 13 shows that the small centrosphere is very faintly outlined and that the centrosome is very small. There can be no doubt that rays begin at the small centrosphere and continue through the outer centrosphere and beyond; in fact the outer centrosphere is limited by a very faint outline, which does not interrupt the course of the fibres. There appear to be vacuolations within the space enclosed by the irregular outline of the outer centrosphere, a condition which is not well shown in the figure. The outline itself is not a distinct membrane, but on the contrary marks the extreme limit of what seems to be a progressive vacuolation, which advances outward in all directions except toward the animal pole. Neither in the first nor in the second polar cell of the specimen from which Figure 13 was drawn could any trace of a centrosome or centrosphere be found.

The question of the fate of the deep centrosome of the second maturation spindle involves the question of the origin of the first cleavage spindle. The principal part of the discussion of the latter question I shall leave for another division of this paper. There are, however, certain points which may be considered here.

Unlike the egg-nucleus in sea-urchins and tunicates,[1] the egg-nucleus in gasteropods moves but slightly from the region where the polar cells are formed. Hill ('95) has shown for Sphærechinus that while the egg-nucleus is in the resting stage at the centre of the egg, it has no rays indicating the presence of a centrosome. The sperm-nucleus, with its centrosome, may be seen at this time a sufficient distance away from the egg-nucleus to enable one to determine readily the relationship of the single aster present in the egg. The case with gasteropods is quite different. Not only is the egg-nucleus eccentric in position, but the astral rays belonging to it persist till a very late stage in the development of the two nuclei. Kostanecki und Wierzejski find for Physa that as the sperm-nucleus, with the aster in advance, moves toward the egg-nucleus, the astral rays of the egg-nucleus begin to disappear, as if they were being assimilated by the sperm-aster, while the two are still a considerable distance apart. In both Limax and Limnæa I have seen quite the opposite conditions and have never seen the phenomena these authors describe. Instead of a diminution of the area affected by the deep aster of the second maturation spindle, I find that, as the egg-nucleus develops, the centrosphere enlarges and the extent of the rays

[1] The statement with regard to the movement of the egg-nucleus in tunicates is based on unpublished evidence, which Dr. H. E. Crampton of Columbia University has kindly permitted me to refer to.

becomes greater (Plate 3, Figure 20). I have never seen for Limnæa the stage shown for Limax in Figure 20. Although every indication of an egg-aster finally disappears, the time of its disappearance is very much delayed as compared with many other animals.

There are many interesting questions which arise in connection with the disappearance of the egg-centrosome. There are difficulties in proving either that the disappearance is permanent or that it is only temporary. If one maintains that the centrosome after the formation of the second polar cell simply goes into a resting stage, and thus becomes invisible, but finally reappears, the only answer that can be made is, that such a statement is an assumption that can neither be proved not disproved. It is an easy matter to find small dense bodies in the region of the egg-nucleus, and even bodies surrounded by radiations. Often many of these small dense bodies may be found in such positions with reference to the two nuclei in the egg as to seem to be significant, but the difficulty comes in deciding which, if any, of these many centrosome-like structures are really centrosomes. For, not only may these structures be found in close relation to the nuclei, but similar appearances are frequent throughout the egg. With such difficulties as these to contend against, it is of the greatest importance that the phases in the regressive metamorphosis of the egg-centrosome be followed with the closest scrutiny.

III. The Nucleus.

In my original plan of work I was little concerned with the nucleus, but as the investigation progressed two problems of great interest and importance claimed my attention. These are: first, the relation of the nucleoli or karyosomes to the chromatin in the resting nuclei, and, secondly, the question of the reduction division in the Roux-Weismann sense; that is to say, a reduction of qualities by a transverse division of the single chromosomes in the second maturation spindle. I shall take up the second of these questions first, because it comes first in the stages of development which I have studied.

1. Division of Chromosomes.

The apparent variation in the number, size, and form of the chromosomes in the maturation spindles of Limax maximus made the study of these elements a particularly difficult and perplexing one. As long as I worked exclusively with this material, the possibility of ever being able

to find evidence concerning a reduction division in the second maturation spindle seemed to me very remote. The number of chromosomes in the first maturation spindle of Limax maximus varies from sixteen to twenty, and sometimes twenty-one or twenty-two of them may be seen. Platner ('86) and Garnault ('88) found for Arion sixteen to twenty chromosomes; but it is quite possible, as Boveri ('90) suggests, that the normal number is really sixteen, — a number which seems to be typical for gasteropods. The fact that in Limax maximus the chromosomes exhibit such variation in size and form, leads me to believe that in the telophase of the first maturation spindle of this species, and possibly of Arion also, not all the bodies seen are simple chromosomes, but rather that some of them are the result of an appreciable separation of the elements composing the "dyads," so that, while some of the supposed chromosomes are unseparated dyads, others are simply one of the components of a dyad. I believe the tetrad formation of the chromosomes to be characteristic of the prophase of the first maturation spindle. When the elements of the dyads are very close together, the resulting appearance is that of a very large chromosome, much larger than the smaller ones. The smaller chromosomes occur most frequently near together, either in pairs or suggesting a paired arrangement. That they are actually joined together, I could not demonstrate satisfactorily. There are cases, however, in which it is a matter of considerable doubt whether a mass of chromatin is a single body with a constriction at the middle, dumb-bell fashion, or whether there are really two chromosomes very near together. In one case I was able to make out the sixteen dyads very distinctly in the polar cell (Plate 3, Figure 17), but the chromosomes remaining in the egg were so closely massed that it was impossible to count them. A curious condition is to be noticed in Figure 21. The number of dyads in the polar cell is fourteen, and the number of chromosomes remaining in the egg is also fourteen. This is quite an unusual variation, and it is probable that two chromosomes at either end of the spindle were obscured in some way. The evidence afforded by a single instance, even though as satisfactory as that shown in Figure 17, is alone not sufficient to carry conviction, but taken with the results I have obtained from my study of Limnæa, affords reasonable ground for believing that the explanation which I have offered of the appearance of chromosomes in excess of the number sixteen is the correct one.

The eggs of Limnæa are more favorable for following the phases of the maturation divisions. Material fixed in 3 per cent nitric acid showed

better preservation than that fixed by any other method. The earliest stage of the chromosomes derived from the germinative vesicle that has come under my observation is shown in Plate 1, Figure 2. The outline of the germinative vesicle has only partially disappeared, and the chromosomes are still indefinite in number and unlike in form. Their arrangement on the spindle can hardly be said to have more than begun. I have no stages between this condition and that represented by Figure 4, but I have in other specimens abundant corroboration of the appearance which Figure 4 shows in regard to the method of separation of the chromosomes in the first maturation division (compare Figure 1). In the first maturation spindle I have always found sixteen chromosomes, some of them more or less completely divided, others not only divided but separated a short distance. Examination of Figure 4 will show that some chromosomes are considerably longer than others. The shorter ones lie end to end on the same spindle fibre. These I take to be chromosomes which have completed their separation, and have now begun to move toward their respective poles. At the middle point of the longer chromosomes, which corresponds with the equator of the spindle, there is an "elbow." Chromosomes in which this elbow is less prominent, are longer than those in which it is large. The meaning is quite clear. The appearances seen in the chromosomes of the first maturation division of Limnæa are due to the more or less complete *splitting* and separation of elongated chromosomes.

Leaving now for a time the discussion of the phases immediately following metakinesis in the first maturation spindle, I shall take up the consideration of the chromosomes in the prophase of the second maturation division. Figure 11 (Plate 2) illustrates a condition which represents partly the prophase and partly the metaphase of division: that is to say, some of the chromosomes are undivided and some have just divided. The chromosomes of this spindle are distinctly dumb-bell shaped, and lie with their long axes parallel with the axis of the spindle. Here, as in the first maturation spindle, I find sixteen chromosomes, all of which are arranged on the outer, thicker fibres of the spindle, never, as is usual in Limax, through the axis of the spindle. Careful examination of the successive sections from which Figure 11 was constructed showed that some of the dumb-bell shaped chromosomes were completely divided across the "handle," and that migration from the equator had barely begun. Not only is the form of the chromosomes in the prophase of the second maturation division that of a dumb-bell, but even as early as the telophase of the first maturation division all the chromosomes, both

those in the group that is to go into the first polar cell and those that are to remain in the egg, have this dumb-bell shape. Even in the metakinesis of the first maturation division I have occasionally seen evidence of a transverse constriction of the chromatin rods. Thus we seem to have in Limnæa a partially concealed "tetrad formation." The presence of the transverse constriction so soon after the longitudinal splitting leaves little doubt that the division as it finally takes place in the second maturation spindle agrees in position with the early constriction.

This observation does not accord with the results Boveri ('90) obtained for Carinaria, one of the heteropods. He found the chromosomes of the first maturation spindle to be quadruple. This quadruple group splits longitudinally in the first maturation division, and then, after the rearrangement of the chromosomes on the second maturation spindle, the division takes place by a longitudinal splitting, exactly as in the first maturation spindle. Naturally one is disposed to ask, What, then, is the meaning of the quadruple groups?

The thing of importance now to be decided for Limnæa is the manner of formation of these "Vierergruppen" or tetrads. To make sure of an exact answer to that question a study of the processes going on in the rearrangement of the chromatin in the germinative vesicle in preparation for the maturation divisions would be necessary. If the tetrads are formed by two longitudinal splittings of segments of the original spireme thread, as in Ascaris (Boveri, '87), and if the two pairs of elements composing the tetrad are elongated and pressed closely together, as at least they seem to have been in the case of Carinaria, then the two maturation divisions would be "equation divisions," and not reducing, except in the sense of a quantitive reduction. If, on the other hand, the length of the masked tetrads represents the length of the original spireme which, in breaking up, first divides longitudinally and next transversely, then the second maturation division is a reduction division in the Roux-Weismann sense. Apart from the evidence which a study of the early stages in the formation and fission of the spireme thread would give, I have little hesitation in holding that we have in Limnæa a reduction division of the Roux-Weismann type, because soon after the evident longitudinal splitting of the chromosomes there occurs a transverse constriction in each of the resulting halves, which continues until there is complete separation of each half into two parts, which then move toward their respective poles.

2. Nucleoli, or Karyosomes.

After the formation of the second polar cell the egg-nucleus becomes vacuolated, and in most of the cases that I have observed the sperm-nucleus also becomes vacuolated at the same time. Both contain many nucleoli or karyosomes varying in size and situated either at the crossing of linin fibres or arranged along such fibres in bead-like fashion. The nucleoli frequently stain with varying intensity in the same nucleus, but generally the staining of all nucleoli is very faint. The larger ones appear to be vacuolated. I have no doubt of the integrity of these karyosomes as distinct elements. Platner ('86) believed them to be distinct elements; but the opposite view was maintained by Boveri ('90, p. 357), who is inclined to think that Platner's "Karyosomen" are artefacts produced by a crowding together of net-knots and numerous large achromatic nucleoli by an unfavorable method of preservation, and that the mistaken interpretation is in part due to the appearance given in very thin cross-sections. I believe, however, that the structures in question can be shown to actually exist as distinct normal bodies, not artefacts. Moreover, I can bring observations to support Platner's claim that the chromosomes which are taking shape for the first cleavage spindle are to be seen surrounding karyosomes as rings. Platner ('86, p. 53) says, "Die Karyosomen nur erscheinen auf den ersten Blick *völlig* farblos, eine aufmerksame Beobachtung lehrt aber dass die Chromatinsubstanz welche anfangs diffus in ihnen vertheilt war, sich in der Form kleiner Körnchen an der Peripherie concentrirt hat."

"Diese Chromatinelemente sind anfangs noch sehr klein und in grösserer Anzahl vorhanden und entziehen sich dann leicht der Beobachtung. Später sammeln sie sich aber zu einigen wenigen grössern Körpern an und treten dann deutlich hervor. Von diesen Chromatinkörnern enthält die Mehrzahl der Karyosomen zwei Stück, welche meist an zwei diametral gegenüberliegenden Punkten der Peripherie gelegen sind. In den kleinern erkennt man zuweilen nur ein solches Element."

I have not studied the karyosomes sufficiently either to confirm or deny Platner's quoted statement, that the chromatin is diffused through the karyosomes preceding the time of its accumulation into a ring on the periphery. I should say, however, that the fact that the karyosomes show staining reaction before the chromatin is collected on the periphery, and that afterwards they do not (a statement which I have not quoted), is not absolute proof that the chromatin is contained in the substance of the karyosomes. The variations in the stainability of these

bodies may, it seems to me, be due to causes which have nothing to do with the movement of the chromatin. It is a well known fact that the centrosome goes through certain chemical phases, in which it does not stain at all by a method which at other times stains it deeply. It seems quite possible that the karyosomes may also have chemical phases independent of any physical accumulation of chromatin.

Whether the chromatin in the resting stage of the nucleus is distributed through the substance of the karyosomes, or whether instead it is distributed through the nuclear sap, as Klinckowström ('97) thinks is the case with Prostheceræus, I believe Platner is right in saying that chromatic substance is to be found at one period in the resting stage of the nucleus collected in rings about the karyosomes. I have frequently noticed densely staining rings, irregular in outline, surrounding faintly stained karyosomes. My best evidence concerning the points in question is shown in Plate **1**, Figure 6. This figure represents the formation of the first cleavage spindle, and, quite exceptionally, the two so-called pro-nuclei have fused. Rays from each aster have extended into the substance of the fused nuclei, one bundle of rays running nearly through the nuclei. Scattered along this bundle of fibres are numerous deeply stained, bent rods. Similar rods may be seen here and there in the nucleus, apparently unattached. Still others are found lying very close to the faintly stained karyosomes and in more or less intimate contact with them. The chromatin particles surrounding the karyosomes, those lying loose in the nuclear sap, and those being drawn along the penetrating astral rays, are so evidently of the same origin that investigation of the history of the chromatin within the resting nucleus would involve an examination of the changes in the so-called nucleoli or karyosomes.

B. FERTILIZATION.

I. The Early History of the Spermatozoön in the Egg.

1. General Description.

As I stated at the beginning of this paper, the first evidence of the presence of the spermatozoön in the egg of Limax that I have seen, was in specimens taken from the uterus. Not only was the first evidence of penetration of the spermatozoön seen in uterine eggs, but in *all* uterine eggs that I have examined penetration had taken place. Whenever I have found eggs in the oviduct (L. agrestis), great numbers of sperma-

tozoa have always been present surrounding the egg and completely filling the oviduct. This condition suggests simultaneous movement of the eggs and spermatozoa from the hermaphrodite gland. I have never found either eggs or spermatozoa alone in the oviduct.

Under these conditions one might expect self-fertilization, and I have looked carefully for evidence of it. That self-fertilization has not taken place, at least in the oviducal eggs that I have examined, may be explained by what seems to be a fact, viz. that the spermatozoa occupying the oviduct are in an immature condition. In the developing sperm element, as the head of the spermatid begins to take on the form characteristic of the mature condition, the tail is continually growing in length. The end of the tail of the spermatid exhibits a large knob. In the fully developed free spermatozoön there is no indication of this knob. Now, in the oviducal spermatozoön of Limax agrestis I have seen this knob-like structure and I am inclined to think all the spermatozoa in the oviduct are in this immature condition.

So far as I know, the spermatozoön has never been observed in the act of penetrating the egg in gasteropods. It is not very important, however, in gasteropods that this phenomenon should be observed, since the tail in following the head into the egg affords the observer the means of determining the topographical relations necessary for noting certain preliminary processes of fertilization.[1]

In the case of Limax maximus I have found, in one instance, a spermatozoön very near the periphery of the egg, which it apparently had but recently penetrated, while the first maturation spindle was migrating to the periphery to form the first polar cell (Plate **3**, Figure 16). When, in other instances, the spermatozoön was in practically the same stage as represented in the figure just mentioned, I have found the first polar cell completely formed (Plate **3**, Figure 21). In one instance (L. agrestis, Plate **3**, Figure 14) I found the spermatozoön with head and tail still connected, and the germinative vesicle of the egg just disappearing. Kostanecki und Wierzejski ('96) found in Physa that a spermatozoön may penetrate the egg and a large sperm-aster may be developed by the time the first maturation spindle is formed. On the other hand, they found that, in the same species, the spermatozoön may not penetrate the egg until both polar cells have been formed.

[1] I may anticipate criticism of this statement by calling attention to what is probably a fact, that the viscidity of the egg-cytoplasm prevents *great* whiplash movements of the spermatozoön; it is not likely therefore that the tail is "fixed" in a position far from the path along which the spermatozoön has progressed.

A very suggestive phenomenon in the early history of the spermatozoön in the egg is shown in Plate 3, Figure 15. It seems as if the attraction which caused the spermatozoön to penetrate the egg were only a general attraction, and not located in a definite region of the egg; for after penetration the head made almost a complete circle before it came within the more definite influence of the egg-centrosome, or before the egg-centrosome and the sperm-centrosome had entered upon the proper phases for attracting each other. At other times, as in Figure 14, the spermatozoön on entering moved straight ahead, not stopping until it had come in contact with the membrane on the other side of the egg. Here it would have remained until the head and tail had separated.

In a preceding paragraph I have referred to the preliminary processes through which a spermatozoön in the egg may be said to go. These processes are: first, the change in form of the sperm-head; and, secondly, the separation of the head from the tail. I shall describe these processes in turn.

2. Changes in the Sperm-Head.

The sperm-head in its fully developed condition, and before it has entered the egg, has the form of a skewer with one, more or less complete, spiral turn. The sperm-head in Figure 15, although broken away from the tail, still retains the general form it had before it entered the egg. As a rule, however, immediately after penetration it undergoes a modification in form. The nature of this modification is well shown in Figure 14. The fact that the head is still attached to the tail, enables one to see that, in this case at least, the long axis of the head is now at right angles to its original long axis. Kostanecki und Wierzejski ('96, Figure 1) show the same condition for Physa. Apparently the change begins very soon after the spermatozoön enters the egg, for in only two instances (one of them shown in Figure 15) have I seen the normal form preserved. It also seems probable that the sperm-head, after becoming elliptical, does not increase in size for a considerable time, for many sperm-heads are found having the same elliptical form, and apparently the same size, both when they are at the periphery of the egg (Plate 1, Figure 1, Plate 2, Figure 8, and Plate 3, Figure 17) and when advanced in their course toward the egg-aster (Plate 3, Figure 21). This fact is brought out better by the figures of Kostanecki und Wierzejski than by mine. I do not believe any especial significance can be attached to this change in the form of the sperm-head. At first thought,

the comparison of the sperm-heads in Figures 14 and 21 would seem to lead to the conclusion that the centrosome in the egg represented in Figure 21 had moved from the side to the end of the head (if the change in form shown in Figure 14 is typical); but such a change in position it seems to me would be meaningless. The difference between the two may be explained by the fact that the sperm-head would naturally present the smallest surface while being drawn through the yolk granules and the protoplasm.

Besides the modification of the sperm-head, there is another phenomenon which, like the one just described, is incidental rather than essential. I refer to the early appearance of a clear area about the sperm-head. The conditions are represented in Figures 14, 15, 21, etc. The outer limit of this area is usually marked by a fine sharp line, so that the whole has the appearance of a clearly defined vacuole, with the sperm-head at the centre. One can make out fine threads radiating from the sperm-head to the margin of the vacuole. In the eggs that I have examined there is no visible modification of the form of the sperm-head by these radiating threads in the vacuole, except in two cases. One of these is represented in Figure 15, where I find that the radiating threads have caused a modification of the outline of the sperm-head. Wherever a thread comes in contact with the sperm-head, a projection, apparently from the head, is formed. The origin and meaning of these radiating threads seem to me of considerable interest in connection with the subsequent changes in the sperm-nucleus, and hence I shall discuss that subject in the next division of this paper.

3. The Sperm-Head and the Beginning of the Sperm-Centrosome.

The change of most importance in the early history of the spermatozoön in the egg is the breaking away of the sperm-head from the tail. The interval of time between the penetration of the sperm-head and its separation from the tail may be short or long according as we interpret the conditions shown in the figures I have made. In some cases it appears probable that the spermatozoön has travelled over a considerable part of the substance of the egg before the head is separated; in others (Figures 1, 7, 8, 11, 16, 17, etc.) either the sperm-head on entering has left the tail outside, or the tail, though entering, has been resorbed. However, the *time* of separation of the head from the tail is not so important as the *manner* of separation. It is to the manner, and still more to the *cause*, that I desire now to direct attention.

Kostanecki und Wierzejski in their Figure 1 give a stage of an egg of Physa which is slightly more advanced than my Figure 14. The head in their figure, as in mine, is represented as being still attached to the tail. Between the head and the stainable tail there is the so-called middle piece, containing the densely stained body supposed to be the centrosome. I have tried to demonstrate the existence of a middle piece and a centrosome in the specimen from which my figure was drawn. Although the sperm-head and a portion of the tail lie in a single section, I am unable to make out any differentiation in the tail near the point of union with the head, the only modification of the tail at that point being a slight swelling. Unfortunately I have but this single specimen showing the head still connected with the tail.

In the work of Wilson and others on the changes of the spermatozoön in the egg, it has been established that the centrosome, in many animals at least, lies in the middle piece of the spermatozoön, and the discovery of any granule within the middle piece has come to be considered as the discovery of a centrosome. Waiving the question of the existence of a differentiated middle piece in the spermatozoön of gasteropods, there are proofs, beside those brought forward by Kostanecki und Wierzejski, that a centrosome exists near the base of the sperm-head. Wilson and Mathews ('95) among others have shown that the sperm-head soon after penetration turns so that its basal end is nearest the egg-aster. At the time of turning a small aster is visible at the base of the sperm-head. An important point in the turning of the head is, that the centrosome is located at the base of the sperm-head. Now, if the tail is present in the egg, it serves as a landmark to show when the turning of the sperm-head takes place and how great is the angle through which the axis of the sperm-head passes. In sea-urchins the tail does not enter the egg with the sperm-head and centrosome; hence the degree of turning cannot be noted as accurately as is possible in the eggs of gasteropods. The difficulty with gasteropod spermatozoa, however, is that because of the early change in the form of the sperm-head and its separation from the tail, the observer is unable to distinguish apex from base. Fortunately this difficulty does not exist in two of the series of preparations that I have. Figure 14 illustrates one of them. In this case the head and tail are still attached. In the other case (Figure 15) the form of the detached sperm-head is very nearly the same as that of a mature sperm-head before penetration. Whatever movements the tail may have gone through while still attached to the head, it is fair to assume that the position of the tail shown in the

drawing (Figure 15) is the position it had when the sperm-head separated from it. In this series of sections, and in another of mine similar to it, the base of the sperm-head is turned toward the egg-nucleus; in the section represented in the drawing (Figure 15) the turning has been through an arc of 90 degrees. The fact that in both series the base is turned toward the egg-aster shows that the force which caused the turning must have acted at the base. The difficulty comes now in finding the structure in which that force resides. I have said that I have been unable to find a centrosome in the series represented in Figure 14, and I am not certain of having seen it even in the preparation from which Figure 15 was drawn. However, from what we know of the turning of the sperm-head in other forms, we can assume that the centrosome exists in this case, even if it is not visible. I have shown in the figure two structures which *may* be centrosomes. Extending from the base of the sperm-head in advance and to the left of it I have shown two fibres, thicker than the others that surround the sperm-head, each containing at its middle point a minute granule. On examination of the specimen with a $\frac{1}{18}$ inch homogeneous Zeiss lens these two fibres are seen to be composed of numerous very fine fibrils. This recalls the condition that Kostanecki ('96, Figure1) shows for the free sperm-head of the sea-urchin. In the sea-urchin, however, there is only one bundle of rays, and that extends from the base directly toward the centre of the sperm-aster. In the other series (not figured) of the same stage as the one represented in Figure 15, there is evidence of a granule in a bundle of fine fibres which extends from the base of the sperm-head. Fibres can also be seen radiating from this granule into the surrounding vacuole, but these radiations have no connection, as far as I can determine, with the more prominent threads extending from the sperm-head through the vacuole. Since in Figure 15 — a stage in which the centrosome is known to be active — there is no evidence of connection between a centrosome and the fibres radiating from the sperm-head, the radiating threads about the sperm-head shown in Figure 14 can hardly be said to be due to the influence of a centrosome.

The evidence I have of the existence in the egg of a structure which I can say positively is a sperm-centrosome in its earliest stages is very meagre. I have seen in the egg many appearances in close proximity to the free sperm-head which suggested an aster, but examination in other parts of the cell afforded many examples of similar ill-defined aster-like structures. In Figure 21 I have drawn a structure that may be the sperm-aster in an early condition; it lies within the vacuole of

the sperm-head at the end nearest the egg-aster. The fact that it lies on the side of the sperm-head which is directed toward the egg-aster is, I believe, a strong argument that this particular structure is the sperm-aster. The discussion of examples of undoubted sperm-asters I shall leave for the next sub-division of this paper.

II. The Sperm-Centrosome.

Kostanecki und Wierzejski ('96) have shown that after the spermatozoön has entered the egg and the sperm-head has taken on a spherical form, the centre of activity is within the sperm-aster. These authors have also shown that at the earliest appearance of the sperm-aster in the egg it is very small and near the sperm-head. As the aster increases in size, it is found sometimes at a considerable distance from the sperm-head. Later in the progress of development the sperm-aster and sperm-head (the latter now considerably vacuolated) are found near together moving toward the egg-aster, the sperm-aster leading the way. Frequently, even at a very early stage, the sperm-centrosome divides into two parts, and a gradually developing spindle forms between them.

It has been supposed generally that the sperm-aster in its course toward the egg-aster takes a position somewhere between the egg-aster and the sperm-head. Some of the figures by Kostanecki und Wierzejski show exceptions to this. For example, in their Figure 3, showing the first maturation spindle at the top of the figure, the sperm-head lies to the left near the periphery of the egg, but the sperm-aster is below the centre. Likewise in their Figure 14, the sperm-aster, instead of lying between the sperm-nucleus and the deep end of the second maturation spindle, is situated so that the line joining it to the sperm-nucleus makes a right angle with the line uniting sperm-nucleus and the inner end of the second maturation spindle. At first I was inclined to think that a second sperm-head in the egg had escaped their observation; but since that time I have found that in my own material it is far easier to make out sperm-nuclei than sperm-asters. Furthermore, I am able to corroborate for Limnæa (Figure 7) some of the conditions which they have shown for Physa. I have no important additions to make to the accounts of these authors on the early changes of the sperm-centrosome in the egg. The facts I have collected are all corroborative of the results obtained by Kostanecki und Wierzejski. I shall therefore describe my observations only briefly.

The sperm-nuclei shown in Figures 5 and 7 are pointed on the side

nearest the sperm-aster. This condition was first noticed by Mark for Limax campestris in 1881. In Figures 13, 20, 29, of Kostanecki und Wierzejski are shown striking modifications in the form of the egg-aster and sperm-aster. Apparently each aster is repelled or possibly, as Kostanecki und Wierzejski suggest, is being assimilated by the other. The interesting facts shown in these figures and in my own Figures 2 and 7 are: first, in each of the five cases the process of maturation is not completed, and, secondly, there *has been* an attraction existing, else the sperm-aster and the egg-aster never would have come as near together as they have. The fact that the functions of the egg-aster are not yet completed, may be sufficient to account for the temporary repulsion.

Considerable attention was given in Part A of this paper to the variable form of the centrosome. In the study of the egg-centrosome, the centrosphere afforded a sort of standard for comparison — a very unsatisfactory one, however. The sperm-centrosome has no centrosphere. The condition of the sperm-centrosome in the specimens I have observed which is most nearly typical of the conditions of centrosome structures in general is that shown in Figure 5. The centrosome in that figure is a small dense granule, which is at the point of origin of the astral rays.

The sperm-centrosome shown in Plate **2**, Figure 10 is the largest I have seen. In fact, I imagined when I first saw it that it was a sperm-head with possibly a centrosome beneath it, but I now believe that the dark body seen in close proximity to the radiations from the large centrosome is the sperm-nucleus belonging to it. Another sperm-head is visible in the egg, but it is near the periphery and is still homogeneous, whereas the more central sperm-head gives evidence of being vacuolated (a condition not shown in the figure).

Another condition of the sperm-aster is shown in Figure 2. I have not been able to make out anything in the central region of the aster that has the appearance of a centrosome, there being instead a large, faintly stained, thickly reticulated area, from which astral rays extend. This central region is very much flattened, apparently owing to a repulsion between this aster and the deep aster of the maturation spindle. The repulsion apparent in the astral rays of both these asters is manifest also in the flattened condition of their central reticulated areas. The peculiar distortion and partial displacement of the reticulated area of the deep maturation spindle affords perhaps the strongest evidence I have presented of a repulsion of one aster by another. The centrosomes could be found only in the peripheral aster of the maturation spindle.

III. The Origin of the First Cleavage Spindle.

The fact that a fully formed sperm-spindle exists in the egg while the processes of maturation are going on, does not of itself prove that the sperm-spindle will become the first cleavage spindle; but nevertheless a strong presumption is created that such is the case. If a spindle arising from the division of the egg-centrosome should also be found after the formation of the second polar cell, the reason for the existence of a sperm-spindle could not be easily explained. As far as I know, however, the egg-centrosome has never been found to divide and produce a spindle after the formation of the second polar cell; but that does not end the matter. In the first place, it is impossible to know whether every sperm-centrosome divides to form a spindle; and in the second place, the sperm-spindle, whether formed, or only potentially existing in the sperm-centrosome, nearly disappears from view during the resting stage of the germ-nuclei.

Kostanecki und Wierzejski maintain that they have followed the history of the sperm-centrosome through even this almost quiescent stage to the actual formation of a cleavage spindle. Their Figure 33a represents the centrosome as having nearly disappeared. Even on this evidence, how is one to prove that the egg-centrosome has totally disappeared "because of inability to carry on the division of the cell," while the sperm-centrosome, a new organ, becomes predominant and develops into the first cleavage spindle? If at any time a link is missing in the chain of the history of the sperm-spindle, it seems to me we cannot exclude the possibility that the egg-centrosome may come again to view, and actually take part in forming at least a part of the first cleavage spindle.

In my discussion of the changes of the egg-centrosome, I have said that, although the disappearance of the egg-centrosome and the astral rays is long delayed, they nevertheless come to a condition in which it is impossible to distinguish them from the yolk granules and the protoplasm. I have also said that I have not seen a stage such as Kostanecki und Wierzejski represent in their figures, where the rays of the egg-aster are apparently being assimilated by the rays of the sperm-aster; but I have not seen exactly the conditions of germ-nuclei which these authors find at the time assimilation is going on. However, their figures as a whole do not bear out the "assimilation" theory completely, as, for example, in Figure 27, where the egg-aster (even though the germ-nuclei are nearer together than in Figure 25 or 28) is still appar-

ently active. When we come to the stage represented in their Figure 33a, and still further to conditions such as I have found where no aster can be detected in connection with either egg-nucleus or sperm-nucleus, then we may well question the value of the "assimilation" theory.

I have referred in an earlier part of this paper to the clear evidence brought forward by Hill ('95) to show that in the egg of Sphærechinus the egg-aster completely disappears. The sperm-aster, from the time it appears at the base of the sperm-head till the first cleavage spindle is formed, is clearly visible; and the sperm-centrosome and its product, the sperm-spindle, are accompanied by the only astral structures that are visible. Fick ('93) finds the first cleavage spindle to be of spermatic origin, but the proof to be found in the existence of an "archoplasmic" mass is not so satisfactory as the evidence produced by Hill.

Some of the most recent work on the history of the centrosomes in the egg tends to re-open the question of the origin of the first cleavage spindle. Foot ('97) maintains for Allolobophora that whatever the evidence of an aster about the spermatozoön in the egg, the cleavage aster comes from the egg itself. According to this investigator, the spermatozoön gives to the developing embryo only the sperm-head. The first cleavage spindle comes from the egg-aster, although that structure is invisible during the resting stage of the egg-nucleus. Klinckowström ('97) finds in a planarian that both egg-aster and sperm-aster disappear during the resting stage of the germ-nuclei. When two asters make their appearance to form the first cleavage spindle, they are at a considerable distance from each other and in such relation to the two germ-nuclei that it is impossible to decide their origin.

The conditions encountered by Klinckowström are apparently the same as those represented in my Figures 12, 18, and 19, referred to above; and since he, as I, could not follow the changes in the centrosome continuously, we can not say, as others have said of centrosomes in practically the same relation to nuclei, that both are of spermatic origin. Hence, if anything leading toward conclusive proof is to be known, it must be learned from facts striking enough to counterbalance the weakness incident to a break in the series of phases through which the centrosomes are seen to pass. I believe I have in Plate 4, Figures 26, 27, and 28, evidence which proves, just at the stage of development when evidence is most conclusive, that *the first cleavage spindle is wholly of spermatic origin.*

The relation of egg-nucleus and sperm-nucleus in the egg of Limnæa is so constant that the sperm-nucleus is never found between the polar

cells and the egg-nucleus. Frequent observation of the stages in the development of the sperm-nucleus enables one to know, even by a hasty examination of the series of sections, which nucleus is the sperm-nucleus. When, therefore, we find, as in Figure 27, that the sperm-nucleus, although partly overlying the egg-nucleus, is nearer to the two centrosomes, and indeed, between the egg-nucleus and the centrosomes, we know that both centrosomes more definitely belong to the sperm-nucleus than to the egg-nucleus. More important than the position of the centrosomes with reference to the sperm-nucleus, and still dependent on that position, is the fact that the astral rays from either centrosome have extended toward the sperm-nucleus; and, having touched the nuclear membrane, have caused indentations in it, and have even penetrated the nucleus itself and united to form a spindle there. The most careful scrutiny of the sections through the egg-nucleus fails to reveal the slightest trace of its being affected yet by the presence of the asters.

Although many changes take place in the nucleus between the stages represented in Figures 27 and 26, the formation of the cleavage spindle has proceeded just far enough to show that the process of first involving the sperm-nucleus in the spindle continues without interruption until that nucleus is wholly within the spindle, while the egg-nucleus is still outside of it. The series of changes leading up to the formation of a perfect first cleavage spindle with the chromosomes in their prophase position is nearly completed in the stage represented in Figure 28.

In conclusion I desire to answer an objection that may possibly be raised and then to call attention to an important additional fact. It may be urged that at least one of the centrosomes represented in each of the Figures 12, 18, and 19 may still be the egg-centrosome, since it is as near to the egg-nucleus as it is to the sperm-nucleus. In reply to this possible contention, I should say, that, while the various positions which the two nuclei *may* occupy with reference to each other, would easily bring one centrosome into a situation where it would be as near to the egg-nucleus as to the sperm-nucleus, nevertheless such a position in the light of the subsequent history of the centrosome can be shown to be *temporary* and *incidental*. It must be remembered also that the sperm-centrosome, sometimes single and sometimes divided, precedes the sperm-nucleus as the two move toward the egg-nucleus in the early stages of fertilization. The fact that one and sometimes both centrosomes later migrate from a point between the two nuclei and away from the egg-nucleus toward the sperm-nucleus tends to emphasize the inter-

pretation I have given: — that the centrosomes of the first cleavage spindle are related to the sperm-nucleus alone. Although one centrosome is often found very near the egg-nucleus, and even extends astral rays toward it, I have *never found both centrosomes coöperating to involve the egg-nucleus in a spindle before the sperm-nucleus was involved.* This seems to me a point of vital importance, precluding, as it does, the possibility of even one centrosome, being of other than spermatic origin; for if in Limnæa elodes one of the centrosomes comes from the egg, then the incipient first cleavage spindles would be as likely to involve the egg-nucleus as the sperm-nucleus first.

SUMMARY.

The centrosome and the centrosphere are extremely variable structures, both in size and in their reaction to stains.

In the processes leading to the formation of the first polar cell in Limax maximus, no centrosome is visible. The astral rays apparently begin in the thickened wall of a pale centrosphere.

After the formation of the first polar cell, and also after the formation of the second polar cell, the centrosphere is found to be greatly enlarged. This condition is characteristic of both Limax maximus and Limnæa elodes.

In the egg cell of Limnæa elodes great variation in the condition of the centrosome and the centrosphere is observed. Two facts tend to show that the *centrosphere* is not a permanent organ of the cell: First, the centrosphere is sometimes invisible on account of the increased area at the centre of the aster which reacts to the stain, and, secondly, astral rays beginning in the centrosome continue *through* the centrosphere. The centrosphere then appears to be no more than a region of thickenings in the astral fibres.

The *centrosome* in the first maturation spindle of Limnæa, when it is visible, varies from a very minute granule to a condition in which its diameter is as great as the transverse dimension of the spindle itself, or even greater. In the first maturation spindle of Limnæa the centrosome was invisible in only a few cases, the astral rays usually giving evidence of its existence. The centrosome of the second maturation spindle of Limnæa never appeared as a large structure; the number of preparations, however, was limited.

In both Limax and Limnæa after the formation of the second polar

cell, the disappearance of the egg-centrosome and the egg-aster, though long delayed, is complete.

A reduction division of the chromosomes in the Roux-Weismann sense was observed in the second maturation division of the egg of Limnæa elodes. The process consisted of a *transverse* division of the dyads resulting from the longitudinal splitting of the masked tetrads of the first maturation division.

In fertilization the tail follows the sperm-head into the egg, but later is resorbed by the egg cytoplasm. The sperm-head, after breaking away from the tail begins, under the influence of the sperm-centrosome, to move, with its base in advance, toward the egg-aster. At the beginning of this movement the sperm-centrosome is not visible; later it becomes distinctly visible, and often very large, giving evidence of variations comparable in a measure to the variation in the egg-centrosome of the first maturation spindle of Limnæa.

Occasionally the sperm-centrosome of Limnæa divides into two while on its way toward the egg-nucleus, a process prophetic of the existence of these centrosomes at either pole of the first cleavage spindle.

On account of the fact that in Limnæa both egg-centrosome and sperm-centrosome at certain stages disappear for a time, it is impossible to ascertain the source of the first cleavage spindle by following the history of the centrosomes. However, reasonably satisfactory proof that the first cleavage spindle is wholly of spermatic origin is found in the facts that the incipient cleavage spindle involves the sperm-nucleus first, and that the egg-nucleus, so far as my observations have extended, is never involved in the spindle before the sperm-nucleus is entirely drawn upon it.

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EXPLANATION OF PLATES.

The drawings on Plates 1, 2, and 4 represent a magnification of 575 diameters; those on Plate 3 about 400 diameters. Projection of the image was made by an Abbé camera lucida. All figures except 3, 5, and 9 are composites of structures extending through several, in some cases nine, sections.

PLATE 1.

All Figures are of eggs of *Limnæa elodes*.

Fig. 1. First maturation spindle, telophase. Centrosomes of large size. Sperm-head near vegetative pole. Breaking of membrane at animal pole accidental; the flattening of the egg normal.

Fig. 2. First maturation spindle. Portions of the membrane of germinative vesicle still visible. Form of deep aster of spindle modified by sperm-aster.

Fig. 3. Deep centrosome and aster, to show the rays beginning at centrosome and extending through and beyond the centrosphere.

Fig. 4. First maturation spindle. The sixteen chromosomes (concealed tetrads) splitting longitudinally. Deep and peripheral centrosomes unlike.

Fig. 5. Sperm-aster and sperm-nucleus. Nucleus elongated in the direction of its aster, and pointed. Position of egg-aster indicated by dotted outline.

Fig. 6. Germ-nuclei fused into a single (?) cleavage nucleus. First cleavage spindle forming. Chromosomes being drawn to the spindle by the rays which penetrate the interior of the fused germ-nuclei. Occasional nucleoli show chromosomes attached to them.

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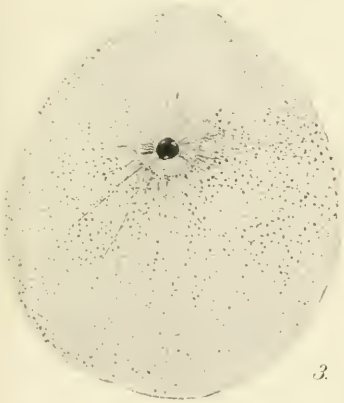

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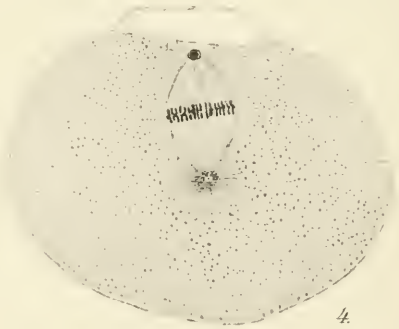

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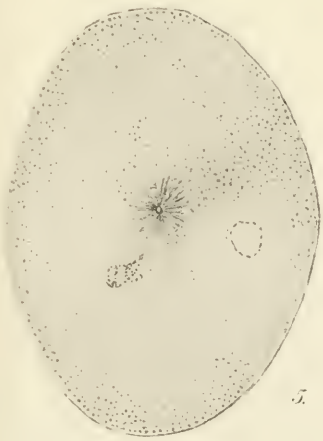

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PLATE 2.

All Figures are of eggs of *Limnæa elodes*.

Fig. 7. First polar cell destroyed. Its position indicated by dotted outline. Enlarging of centrosphere begun. Centrosome of sperm-aster divided into two, but surrounded by a single system of astral rays. Sperm-head pointed on side toward the distant sperm-aster.

Fig. 8. First polar cell; the peripheral centrosphere disintegrating. Deep centrosome surrounded by a small spherical centrosphere; this surrounded, in turn, by an eccentric larger centrosphere.

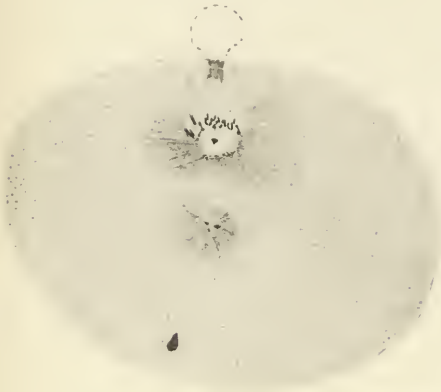
Fig. 9. Transverse section through the peripheral chromosomes of the first maturation spindle. Sixteen chromosomes present.

Fig. 10. To show large sperm-centrosome and, near by, the slightly vacuolated sperm-head. Another sperm-head at periphery of egg. The polar cell in outline projected on the plane of this section. The egg-nucleus is not shown, as it lies in another section.

Fig. 11. Second maturation spindle. Sixteen dumb-bell shaped chromosomes (dyads) about to divide by transverse division. Constructed from successive sections.

Fig. 12. Only one polar cell (second) recognized. Its relation to egg obscured by accidental presence of a foreign body. Formation of first cleavage spindle.

Fig. 13. Telophase of second maturation spindle. Deep centrosphere surrounded by an eccentric outer centrosphere. Sperm-nucleus without sperm-aster.



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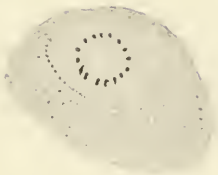

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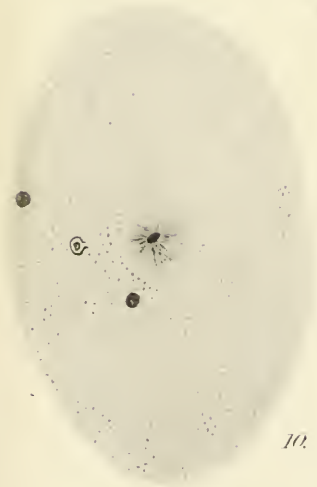

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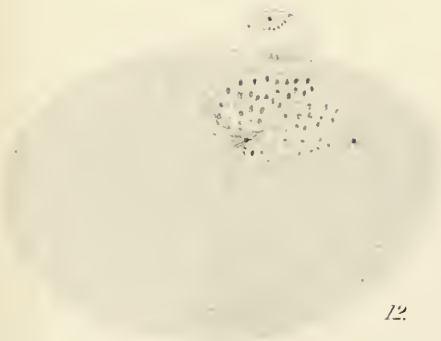

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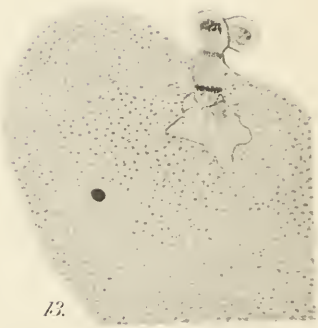

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PLATE 3.

Fig. 14. *Limax agrestis.* First maturation spindle, germinative vesicle having just disappeared. Head and tail of spermatozoön still connected.

Fig. 15. *Limnæa sp.?* First polar cell remains. Second polar cell formed, but broken away from the egg; its "Zwischenkörper" remains near that of the first polar cell. Sperm-head just broken off from the tail, and with base turned toward the egg-nucleus. The sperm-head lies in a vacuole. Two centrosomes (?) may be seen each in a bundle of rays in the vacuole on the side away from the proximal end of the tail.

Fig. 16. *Limax maximus.* Formation of the first polar cell. Centrosphere with thick reticulated wall; no centrosome is visible. Clear area of centrosphere finely reticulated. Sperm-head in lower part of figure.

Fig. 17. *Limax maximus.* First polar cell just formed. Vacuolation or enlarging of centrosphere begun. Two centrosomes (?) in the centrosphere. Sperm-head in usual vacuole. Sixteen dyads in first polar cell.

Fig. 18. *Limax maximus.* Centrosomes about to form the first cleavage spindle.

Fig. 19. *Limax agrestis.* Polar cells lost. First cleavage spindle forming.

Fig. 20. *Limax maximus.* First polar cell lost; second polar cell still united to the egg. The germ-nuclei are nearly surrounded by numerous small bodies resembling centrospheres.

Fig. 21. *Limax maximus.* First polar cell just formed. Enlargement of centrosphere further advanced than in Figure 17. Two minute centrosomes with radiations within the centrosphere. Sperm-head with problematic centrosome within the sperm vacuole Blunt end of tail is the one to which the sperm-head was attached.

Fig. 22. *Limax maximus.* Telophase of first cleavage spindle. Nuclear membrane already formed about the chromosomes. Centrosomes divided in anticipation of the next cleavage.

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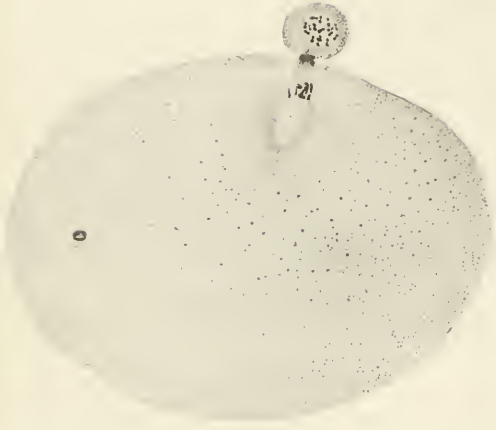

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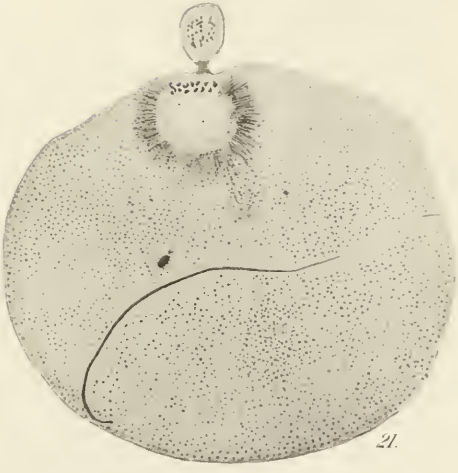

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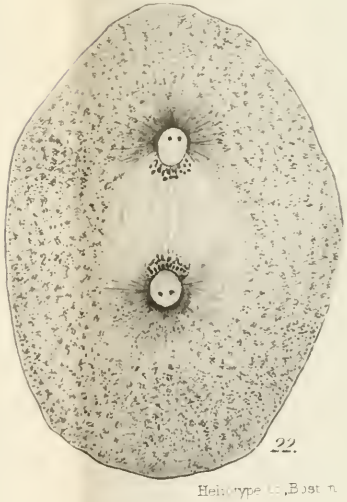


22.

H. L. [illegible]

Heliotype Co., Boston.

PLATE 4.

All Figures, except Figure 25, are of eggs of *Limnæa elodes*.

Fig. 23. First maturation spindle. At either pole of the spindle is collected a mass of precipitate, the whole resembling a so-called centrosphere.

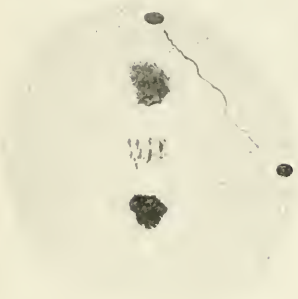
Fig. 24. First maturation spindle. Apparently the centrospheres were in the process of being decolorized when the process of decolorizing was checked.

Fig. 25. *Limax agrestis*. The germinative vesicle contains two nucleoli and "Linin" fibres with elements of future chromosomes arranged in "skein" stage.

Fig. 26. First cleavage spindle. Structures that seem to be the sperm-chromosomes now occupy the middle of the spindle, and the egg-chromosomes are about to be drawn into it.

Fig. 27. First cleavage spindle. Rays near the incipient spindle are beginning to penetrate the sperm nucleus, while the egg-nucleus is still unaffected. The chromosomes are not yet formed.

Fig. 28. First cleavage spindle. The sperm-chromosomes occupy the whole breadth of the spindle, while the egg-chromosomes are at one side of the spindle. The granular funnel beneath the polar cells probably represents the path through which the egg-chromosomes were drawn to the spindle.



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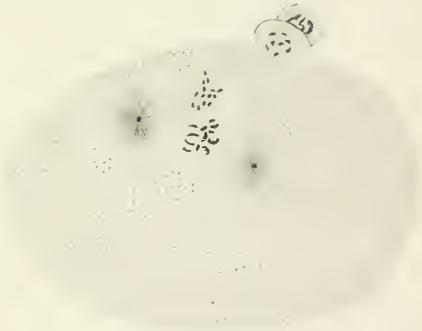

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THE FOLLOWING REPORTS HAVE BEEN PUBLISHED OR ARE IN PREPARATION ON THE DREDGING OPERATIONS OFF THE WEST COAST OF CENTRAL AMERICA TO THE GALAPAGOS, TO THE WEST COAST OF MEXICO, AND IN THE GULF OF CALIFORNIA, IN CHARGE OF ALEXANDER AGASSIZ, CARRIED ON BY THE U. S. FISH COMMISSION STEAMER "ALBATROSS," DURING 1891, LIEUT. COMMANDER Z. L. TANNER, U. S. N., COMMANDING.

A. AGASSIZ. II.[1] General Sketch of the Expedition of the "Albatross," from February to May, 1891.

A. AGASSIZ. The Pelagic Fauna.

A. AGASSIZ. The Deep-Sea Panamic Fauna.

A. AGASSIZ. I.[2] On Calamocrinus, a new Stalked Crinoid from the Galapagos.

A. AGASSIZ. XXIII.[23] The Echini.

JAS. E. BENEDICT. The Annelids.

R. BERGH. XIII.[13] The Nudibranchs.

K. BRANDT. The Sagittæ.

K. BRANDT. The Thalassicolæ.

C. CHUN. The Siphonophores.

C. CHUN. The Eyes of Deep-Sea Crustacea.

S. F. CLARKE. XI.[11] The Hydroids.

W. H. DALL. The Mollusks.

W. FAXON. VI.[8] XV.[16] The Stalk-eyed Crustacea.

ARMAN. XXVI.[26] The Fishes.

ESBRECHT. XVI.[15] The Copepods.

JËS. III.[4] XX.[20] The Foraminifera.

H. J. HANSEN. XXII.[22] The Cirripeds and Isopods.

C. HARTLAUB. XVIII.[18] The Comatulæ.

W. A. HERDMAN. The Ascidians.

S. J. HICKSON. The Antipathids.

W. E. HOYLE. The Cephalopods.

G. VON KOCH. The Deep-Sea Corals.

C. A. KOFOID. Solenogaster.

R. VON LENDENFELD. The Phosphorescent Organs of Fishes.

H. LUDWIG. IV.[5] XII.[14] The Holothurians.

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ROBERT RIDGWAY. The Alcoholic Birds.

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TH. STUDER. X.[10] The Alcyonarians.

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E. P. VAN DUZEE. The Halobatidæ.

H. B. WARD. The Sipunculoids.

H. V. WILSON. The Sponges.

W. McM. WOODWORTH. IX.[9] The Planarians and Nemerteans.

W. McM. WOODWORTH. XXVII.[27] Planktonemertes.

[1] Bull. M. C. Z., Vol. XXI., No. 4, June, 1891, 16 pp.; and Vol. XXIII., No. 1, February, 1892, 89 pp., 22 Plates.

[2] Mem. M. C. Z., Vol. XVII., No. 2, January, 1892, 95 pp., 32 Plates.

[3] Bull. M. C. Z., Vol. XXIV., No. 7, August, 1893, 72 pp.

[4] Bull. M. C. Z., Vol. XXIII., No. 5, December, 1892, 4 pp., 1 Plate.

[5] Bull. M. C. Z., Vol. XXIV., No. 4, June, 1893, 10 pp. [Zool. Anzeig., No. 420, 1893.]

[6] Bull. M. C. Z., Vol. XVI., No. 13, July, 1893, 3 pp.

[7] Bull. M. C. Z., Vol. XXV., No. 1 September, 1893, 25 pp.

[8] Bull. M. C. Z., Vol. XXV., No. 2. December, 1893, 17 pp., 2 Plates.

[9] Bull. M. C. Z., Vol. XXV., No. 4, January, 1894, 4 pp., 1 Plate.

[10] Bull. M. C. Z., Vol. XXV., No. 5, February, 1894, 17 pp.

[11] Bull. M. C. Z., Vol. XXV., No. 6, February, 1894, 7 pp., 5 Plates.

[12] Bull. M. C. Z., Vol. XXV., No. 8, September, 1894, 13 pp., 1 Plate.

[13] Bull. M. C. Z., Vol. XXV., No. 10, October, 1894, 109 pp., 12 Plates.

[14] Mem. M. C. Z., Vol. XVII., No. 3, October, 1894, 183 pp., 19 Plates.

[15] Bull. M. C. Z., Vol. XXV., No. 12, April, 1895, 20 pp., 4 Plates.

[16] Mem. M. C. Z., Vol. XVIII., April, 1895, 292 pp., 67 Plates, 1 Chart.

[17] Bull. M. C. Z., Vol. XXVII., No. 3, July, 1895, 8 pp., 2 Plates.

[18] Bull. M. C. Z., Vol. XXVII., No. 4, August, 1895, 26 pp., 3 Plates.

[19] Bull. M. C. Z., Vol. XXVII., No. 5, October, 1895, 14 pp., 3 Plates.

[20] Bull. M. C. Z., Vol. XXIX., No. 1, March, 1896, 103 pp., 9 Plates, 1 Chart.

[21] Mem. M. C. Z., Vol. XXIII., No. 1, September, 1897, 92 pp., 15 Plates.

[22] Bull. M. C. Z., Vol. XXXI., No. 5, December, 1897, 37 pp., 6 Plates, 1 Chart.

[23] Bull. M. C. Z., Vol. XXXII., No. 5, May, 1898, 18 pp., 13 Plates, 1 Chart.

[24] Bull. M. C. Z., Vol. XXXII., No. 8, August, 1898, 8 pp., 3 Plates.

[25] Mem. M. C. Z., Vol. XXII., No. 2, November, 1899, 116 pp., 22 Plates, 1 Chart.

[26] Mem. M. C. Z., Vol. XXIV., December, 1899, 431 pp., 97 Plates, 1 Chart.

[27] Bull. M. C. Z., Vol. XXXV., No. 1, June, 1899, 4 pp., 1 Plate.

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